

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 November 2002 (07.11.2002)

PCT

(10) International Publication Number
WO 02/088306 A2

(51) International Patent Classification⁷:

C12N

(21) International Application Number:

PCT/US02/11853

(22) International Filing Date:

26 April 2002 (26.04.2002)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/287,539 30 April 2001 (30.04.2001) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/088306 A2

(54) Title: HUMANIZED ANTIBODIES

(57) Abstract: Humanized forms of mouse antibody 3D6 that retain the binding properties of mouse 3D6 are disclosed. Also disclosed are processes for making the humanized antibody, intermediates for making the humanized antibodies, including, nucleotide sequences, vectors, transformed host cells, and methods of using the humanized antibody to treat, prevent, alleviate, reverse, or otherwise ameliorate symptoms or pathology or both, that are associated with Down's syndrome or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy.

HUMANIZED ANTIBODIES

This application claims priority of US 60/287,539, filed 2001 April 30, the entire contents of which are incorporated herein by reference.

The invention relates to humanized antibodies useful for treating and preventing 5 human diseases associated with amyloid β (A β), such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy. Mouse monoclonal antibody 3D6 has been widely used in analytical methods. After 3D6 was administered to a group of 11.5-12 month-old heterozygous, transgenic PDAPP mice (APP V717F) at a weekly intraperitoneal dose of about 10 mg/kg for six months, it has been reported that the mice had significantly 10 reduced plaque burden, although the specific location of the reduction was not disclosed. [Bard, F., et al., *Nature Med.* 6:916-919 (2000); WO 00/72876 and WO 00/72880, 7 December, 2000]. It was asserted that the antibody gained access to the central nervous system in sufficient amounts to "decorate" β -amyloid plaques. Finally, it was stated that mouse 3D6 induces phagocytosis of amyloid plaques in *in vitro* studies.

15 Methods for administering aggregated A β 1-42 to provoke an immunologic response and reduced amyloid deposits are described in PCT publication WO99/27944, published 10 June 1999. The description postulates that full-length aggregated A β peptide would be a useful immunogen. The application also indicates that antibodies that bind to A β peptide could be used as alternate therapeutic agents. However, this appears 20 to be speculation since the supporting data reflect protocols that involve active immunization using, for example, A β 1-42.

WO 99/60024, published 25 November 1999, is directed to methods for amyloid removal using anti-amyloid antibodies. The mechanism, however, is stated to utilize the ability of anti-A β antibodies to bind to pre-formed amyloid deposits (i.e. plaques) and 25 result in subsequent microglial clearance of localized plaques. This mechanism was not proved *in vivo*. This publication further states that to be effective against A β plaques, anti-A β antibodies must be delivered directly to the brain, because antibodies cannot cross the blood brain barrier.

Queen, et al. describe methods of humanizing antibodies [e.g., US Patent Nos. 30 5,585,089, 5,693,761, 5,693,762, 6,180,370].

Humanized forms of 3D6 are needed for use in humans having Down's syndrome, or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy (CAA). However, it is not known whether 3D6 can be humanized so that the humanized antibody retained the binding properties of the mouse antibody.

5 Summary of the Invention

This invention provides humanized forms of 3D6. These humanized antibodies have binding properties (affinity and epitope location) that are approximately the same as those of the mouse 3D6 antibody. The invention includes antibodies, single chain antibodies, and fragments thereof. The invention includes antibodies wherein the CDR 10 are those of mouse monoclonal antibody 3D6 (sequences SEQ ID NO:1 through SEQ ID NO:6) and wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the variable regions have sequences comprising the CDR from 15 mouse antibody 3D6 and specific human framework sequences (sequences SEQ ID NO:7 - SEQ ID NO:10), wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody 3D6. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the light chain is SEQ ID NO:11 and the heavy chain is 20 SEQ ID NO:12.

Also part of the invention are polynucleotide sequences that encode the humanized antibodies or fragments thereof disclosed above, vectors comprising the polynucleotide sequences encoding the humanized antibodies or fragments thereof, host cells transformed with the vectors or incorporating the polynucleotides that express the humanized 25 antibodies or fragments thereof, pharmaceutical formulations of the humanized antibodies and fragments thereof disclosed herein, and methods of making and using the same.

Such humanized antibodies and fragments thereof are useful for, among other things, treating and preventing diseases and conditions characterized by A β plaques or A β toxicity in the brain, such as Alzheimer's disease, Down's syndrome, and cerebral 30 amyloid angiopathy in humans.

The invention also includes use of a humanized antibody of the present invention for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating, preventing, or reversing Alzheimer's disease, Down's syndrome, or cerebral amyloid angiopathy, or
5 to inhibit the formation of amyloid plaques or the effects of toxic soluble A β species in humans.

Detailed Description of the Invention

We have surprisingly found that humanized antibodies, wherein the CDRs originate from mouse monoclonal antibody 3D6 and the framework and other portions of
10 the antibodies originate from a human germ line, bind A β 1-40 and A β 1-42 with at least the affinity with which mouse 3D6 binds A β . Thus, we have a reasonable basis for believing that humanized antibodies of this specificity, modified to reduce their immunogenicity by converting them to a humanized form, offer the opportunity to treat,
both prophylactically and therapeutically, conditions in humans that are associated with
15 formation of beta-amyloid plaques. These conditions include, as noted above, pre-clinical and clinical Alzheimer's, Down's syndrome, and pre-clinical and clinical cerebral amyloid angiopathy.

As used herein, the word "treat" includes therapeutic treatment, where a condition to be treated is already known to be present and prophylaxis - *i.e.*, prevention of, or
20 amelioration of, the possible future onset of a condition.

By "antibody" is meant a monoclonal antibody *per se*, or an immunologically effective fragment thereof, such as an Fab, Fab', or F(ab')₂ fragment thereof. In some contexts, herein, fragments will be mentioned specifically for emphasis; nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody"
25 includes such fragments as well as single-chain forms. As long as the protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred.
30 Antibodies are properly cross-linked via disulfide bonds, as is well known.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 3 or more amino acids.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, et al., J. Mol. Biol. 196:901-917 (1987); Chothia, et al., Nature 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). A humanized immunoglobulin does not encompass a chimeric antibody, having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to

employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Humanized antibodies have at least three potential advantages over non-human and chimeric antibodies for use in human therapy:

- 5 1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).
- 10 2) The human immune system should not recognize the framework or C region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody.
- 15 3) Injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected humanized antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The design of humanized immunoglobulins may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used (acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor immunoglobulin):

- 25 (a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position;
- (b) the position of the amino acid is immediately adjacent to one of the CDRs; or
- 30 (c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, *et al.*, Proc. Natl Acad. Sci. USA 86:10029-10033

(1989), and Co, *et al.*, Proc. Natl. Acad. Sci. USA 88, 2869 (1991)]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

A preferred humanized antibody is a humanized form of mouse antibody 3D6.

The CDRs of humanized 3D6 have the following amino acid sequences:

light chain CDR1:

1 5 10 15
10 Lys Ser Ser Gln Ser Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn
(SEQ ID NO:1)

light chain CDR2:

15 Leu Val Ser Lys Leu Asp Ser (SEQ ID NO:2)

light chain CDR3:

Trp Gln Gly Thr His Phe Pro Arg Thr (SEQ ID NO:3)

heavy chain CDR1:

1 5
Asn Tyr Gly Met Ser (SEQ ID NO:4)

25 heavy chain CDR2:

1 5 10 15
Ser Ile Arg Ser Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val Lys Gly
(SEQ ID NO:5)

30 and, heavy chain CDR3:

1 5 10
Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr (SEQ ID NO:6).

A preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline V_k segment DPK19 and J segment J_k4:

```

 1          5          10          15
40 Xaa Val Val Met Thr Gln Xaa Pro Leu Xaa Leu Pro Val Thr Xaa Gly
      .          20          25          30
        Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
45          35          40          45
        Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
      50          55          60

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Pro Xaa Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 65 70 75 80
 Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 5 85 90 95
 Ser Arg Val Glu Ala Glu Asp Gly Val Tyr Tyr Cys Trp Gln Gly
 10 100 105 110
 Thr His Phe Pro Arg Thr Phe Gly Gly Thr Lys Xaa Glu Ile Lys

Arg

(SEQ ID NO: 7)

15

wherein:

Xaa at position 1 is Asp or Tyr;
 Xaa at position 7 is Ser or Thr;
 Xaa at position 10 is Ser or Thr;
 20 Xaa at position 15 is Leu, Ile, or Val;
 Xaa at position 50 is Arg or Lys;
 Xaa at position 88 is Val or Leu; and
 Xaa at position 109 is Val or Leu.

25 A preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-45 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity:

30 1 5 10 15
 Glu Val Xaa Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Gly
 20 20 25 30
 Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
 35 35 40 45
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 40 50 55 60
 Ala Ser Ile Arg Ser Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
 45 65 70 75 80
 Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Xaa Leu Tyr
 Leu Gln Met Asn Ser Leu Xaa Xaa Glu Asp Thr Ala Val Tyr Tyr Cys

100 105 110
 Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
 5 115
 Thr Xaa Val Thr Val Ser Ser

(SEQ ID NO:8)

wherein:

- Xaa at position 3 is Gln, Lys, or Arg;
- 10 Xaa at position 78 is Ser or Thr;
- Xaa at position 87 is Arg or Lys;
- Xaa at position 88 is Ala, Ser, or Thr; and
- Xaa at position 114 is Leu, Thr, Ile, or Val.

15 A particularly preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK19 and J segment Jk4:

1	5	10	15	
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly				
20	20	25	30	
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser				
25	35	40	45	
Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser				
30	50	55	60	
Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro				
35	65	70	75	80
Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile				
40	85	90	95	
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly				
45	100	105	110	
Thr His Phe Pro Arg Thr Phe Gly Gly Thr Lys Val Glu Ile Lys				
50	Arg	(SEQ ID NO:9).		

55 A particularly preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-45 and J segment JH4: 1

5	10	15
Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Gly		

	20		25		30										
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Gly	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
5															
	35		40		45										
Gly	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
10															
	50		55		60										
Ala	Ser	Ile	Arg	Ser	Gly	Gly	Gly	Arg	Thr	Tyr	Tyr	Ser	Asp	Asn	Val
15															
	65		70		75										80
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Glu	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
20															
	85		90		95										
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
25															
	100		105		110										
Val	Arg	Tyr	Asp	His	Tyr	Ser	Gly	Ser	Ser	Asp	Tyr	Trp	Gly	Gln	Gly
30															
	115														
Thr	Leu	Val	Thr	Val	Ser	Ser									
35															

(SEQ ID NO:10).

A preferred light chain for a humanized antibody of the present invention has the
25 amino acid sequence:

	1	5	10	15												
	Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
		20						25							30	
30	Gln	Pro	Ala	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asp	Ser
		35						40							45	
	Asp	Gly	Lys	Thr	Tyr	Leu	Asn	Trp	Leu	Gln	Gln	Arg	Pro	Gly	Gln	Ser
35	Pro	Arg	Arg	Leu	Ile	Tyr	Leu	Val	Ser	Lys	Leu	Asp	Ser	Gly	Val	Pro
		50					55					60				
	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
40	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Trp	Gln	Gly
		85						90					95			
	Thr	His	Phe	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
45																
		100						105					110			
	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
50																
		115						120					135			
	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
55																
		130			135						140					
	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
60																
		145			150						155					
	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
		165						170					175			
	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu

195	200	205
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser		
5 210	215	
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		(SEQ ID NO:11).

A preferred heavy chain for a humanized antibody of the present invention has the
10 amino acid sequence:

1	5	10	15
Glu Val Gln Leu Val Glu Ser Gly Gly	Gly	Leu Val Gln Pro Gly	Gly
15 20	25	30	
Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly	Phe	Thr Phe Ser Asn Tyr	
35	40	45	
Gly Met Ser Trp Val Arg Gln Ala Pro Gly	Lys	Gly Leu Glu Trp	Val
20 50	55	60	
Ala Ser Ile Arg Ser Gly Gly	Arg	Thr Tyr Ser Asp Asn Val	
65	70	75	80
Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala	Lys	Asn Ser Leu Tyr	
25 85	90	95	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp	Thr Ala Val	Tyr Tyr Cys	
30 100	105	110	
Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp	Tyr Trp	Gly Gln Gly	
115	120	125	
Thr Leu Val Thr Val Ser Ser Ala Ser Thr	Lys Gly	Pro Ser Val Phe	
35 130	135	140	
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly	Gly	Thr Ala Ala Leu	
145	150	155	160
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu	Pro Val	Thr Val Ser Trp	
40 165	170	175	
Asn Ser Gly Ala Leu Thr Ser Gly Val His	Thr Phe Pro	Ala Val Leu	
180	185	190	
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val	Val Val	Thr Val Pro Ser	
195	200	205	
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys	Asn Val Asn His	Lys Pro	
50 210	215	220	
Ser Asn Thr Lys Val Asp Lys Lys Val Glu	Pro Lys Ser Cys	Asp Lys	
225	230	235	240
Thr His Thr Cys Pro Pro Cys Pro Ala Pro	Glu Leu	Leu Gly	Pro
55 245	250	255	
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys	Asp Thr	Leu Met Ile	Ser
260	265	270	
Arg Thr Pro Glu Val Thr Cys Val Val Val	Asp Val Ser His	Glu Asp	

	275	280	285	
	Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn			
5	290	295	300	
	Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val			
	305	310	315	320
	Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu			
10	325	330	335	
	Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys			
	340	345	350	
15	Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr			
	355	360	365	
	Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr			
20	370	375	380	
	Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu			
	385	390	395	400
	Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu			
25	405	410	415	
	Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys			
	420	425	430	
30	Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu			
	435	440	445	
	Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly			
35	Lys		(SEQ ID NO:12)	

Other sequences are possible for the light and heavy chains for humanized 3D6. The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least one chain comprising one or more mouse complementarity determining regions functionally joined to human framework region segments.

In another aspect, the present invention is directed to recombinant polynucleotides encoding antibodies which, when expressed, comprise the heavy and light chain CDRs from an antibody of the present invention. Exemplary polynucleotides, which on expression code for the polypeptide chains comprising the heavy and light chain CDRs of monoclonal antibody 3D6 are given herein. Due to codon degeneracy, other polynucleotide sequences can be readily substituted for those sequences. Particularly preferred polynucleotides of the present invention encode antibodies, which when expressed, comprise the CDRs of SEQ ID NO:1 – SEQ ID NO:6, or any of the variable regions of SEQ ID NO:7 – SEQ ID NO:10, or the light and heavy chains of SEQ ID NO:11 and SEQ ID NO:12.

The polynucleotides will typically further include an expression control polynucleotide sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host cell line, the host cell is propagated under conditions suitable for expressing the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), using any of a variety of well known techniques. Joining appropriate genomic and synthetic sequences is a common method of production, but cDNA sequences may also be utilized.

Below is a cDNA sequence (SEQ ID NO:17), from which the light chain having the amino acid sequence of SEQ ID NO:19 may be expressed.

20	ATGATGAGTCCTGCCAGTCTCCTGTTCTGTTAGTGCTCTGGATTGGAAACCAACGGT 1 -----+-----+-----+-----+-----+-----+-----+ 60 M M S P A Q F L F L L V L W I R E T N G
25	GATGTTGTGATGACCCAGTCTCCACTCTCCTGCCTGTTACCCCTGGACAACCAGCCTCC 61 -----+-----+-----+-----+-----+-----+-----+ 120 D V V M T Q S P L S L P V T L G Q P A S
30	ATCTCTTGCAAGTCAGTCAGAGCCTCTTAGATAGTGATGGAAAGACATATTGAATTGG 121 -----+-----+-----+-----+-----+-----+-----+ 180 I S C K S S Q S L L D S D G K T Y L N W
35	TTGCAACAGCGCCCAGGCCAGTCTCCAAGACGCCATACTATCTATCTGGTGTCTAAACTGGAC 181 -----+-----+-----+-----+-----+-----+-----+ 240 L Q Q R P G Q S P R R L I Y L V S K L D
40	TCTGGAGTCCCTGACAGGTTCTCTGGCAGTGGATCAGGGACAGATTACACTGAAAATC 241 -----+-----+-----+-----+-----+-----+-----+ 300 S G V P D R F S G S G S G T D F T L K I
45	AGCAGAGTCGAGGCTGAGGATGTGGAGTTATTATTGCTGGCAAGGTACACATTTCCT 301 -----+-----+-----+-----+-----+-----+-----+ 360 S R V E A E D V G V Y Y C W Q G T H F P
	CGGACGTTCGGTGGAGGCACCAAGGTGGAAATCAAACGTACTGTGGCTGCACCATCTGTC 361 -----+-----+-----+-----+-----+-----+-----+ 420 R T F G G G T K V E I K R T V A A P S V
	TTCATCTCCCCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGCTGCTG 421 -----+-----+-----+-----+-----+-----+-----+ 480

F I F P P S D E Q L K S G T A S V V C L
 481 CTGAAATAACTTCTATCCCAGAGAGGCCAAGTACAGTGGAAAGGTGGATAACGCCCTCAA + 540
 5 L N N F Y P R E A K V Q W K V D N A L Q
 TCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
 541 S G N S Q E S V T E Q D S K D S T Y S L + 600
 10 AGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCAGAA
 601 S S T L T L S K A D Y E K H K V Y A C E + 660
 15 GTCACCCATCAGGGCCTGAGGCTGCCGTACAAAGAGCTAACACAGGGAGAGTGT (SEQ ID NO:17)
 661 V T H Q G L S S P V T K S F N R G E C (SEQ ID NO:19) + 720

Below is a cDNA sequence (SEQ ID NO:18), from which the heavy chain having
 20 the amino acid sequence of SEQ ID NO:20 may be expressed.

ATGAACTTCGGGCTCAGCTTGATTTCCTTGTCTTAAAAGGTGTCCAGTGTGAA + 60
 1 M N F G L S L I F L V L V L K G V Q C E
 25 GTGCAACTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGCTCTGAGGCTCTCC + 120
 61 V Q L V E S G G G L V Q P G G S L R L S
 30 TGTCAGGCTCTGGATTCACTTCAGTAATATGGCATGTCTGGGTCGCCAGGCTCC + 180
 121 C A G S G F T F S N Y G M S W V R Q A P
 35 GGAAAGGGACTGGAGTGGGTGATCCATTAGGAGTGGTGGTAGAACCTACTATTCA + 240
 181 G K G L E W V A S I R S G G G R T Y Y S
 40 GACAATGTAAGGGCCGATTCAACCATCTCAGAGAGAATGCCAAGAACAGCCTGTACCTG + 300
 241 D N V K G R F T I S R E N A K N S L Y L
 45 CAAATGAACAGTCTGAGAGCTGAGGACACGGCTGTCTATTATTGTGTCAGATATGATCAC + 360
 301 Q M N S L R A E D T A V Y Y C V R Y D H
 50 TATAGTGGTAGCTCCGACTACTGGGCCAGGGCACCTGGTCACAGTCTCCTCAGCCTCC + 420
 361 Y S G S S D Y W G Q G T L V T V S S A S
 55 ACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCAAGAGCACCTCTGGGGCACA + 480
 421 T K G P S V F P L A P S S K S T S G G T
 60 GCGGCCCTGGCTGCCTGGTCAAGGACTACTTCCCGAACCGGTGACGGTGTGAAAC + 540
 481 A A L G C L V K D Y F P E P V T V S W N
 TCAGGCGCCCTGACCAAGCGGCGTGCACACCTTCCGGCTGTCTACAGTCTCAGGACTC + 600
 541 S G A L T S G V H T F P A V L Q S S G L
 601 TACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTGGCACCCAGACCTACATC + 660
 Y S L S S V V T V P S S S L G T Q T Y I

TGCAACGTGAATCACAAGCCGACACCCAAGGTGGACAAGAAAGTTGAGGCCAAATCT
 661 C N V N H K P S N T K V D K K V E P K S 720
 TGTGACAAAACTCACACATGCCACCAGCACCTGAACTCTGGGGGACCGTCA
 721 C D K T H T C P P C P A P E L L G G P S 780
 GTCTTCCCTTCCCCAAAACCCAAGGACACCCATGATCTCCGGACCCCTGAGGTC
 781 V F L F P P K P K D T L M I S R T P E V 840
 ACATGGCTGGTGGTGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTG
 841 T C V V V D V S H E D P E V K F N W Y V 900
 GACGGCGTGGAGGTGCATAATGCCAAGAACAGCCGGGAGGAGCAGTACAAACAGCAGC
 901 D G V E V H N A K T K P R E E Q Y N S T 960
 TACCGTGTGGTCAGCGTCCTCACCGTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTAC
 961 Y R V V S V L T V L H Q D W L N G K E Y 1020
 AAGTGCAAGGTCTCAAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAAGCC
 1021 K C K V S N K A L P A P I E K T I S K A 1080
 AAAGGGCAGCCCCGAGAACACAGGTGTACACCCCTGCCCATCCGGATGAGCTGACC
 1081 K G Q P R E P Q V Y T L P P S R D E L T 1140
 AAGAACCGAGTCAGCCTGACCTGCCCTGGTCAAAGGCTCTATCCAGCGACATGCCGTG
 1141 K N Q V S L T C L V K G F Y P S D I A V 1200
 GAGTGGGAGAGCAATGGGAGCCGGAGAACAACTACAAGACCACGCCCTCCGTGCTGGAC
 1201 E W E S N G Q P E N N Y K T T P P V L D 1260
 TCCGACGGCTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG
 1261 S D G S F F L Y S K L T V D K S R W Q Q 1320
 GGGAACGTCTTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACGCAGAAG
 1321 G N V F S C S V M H E A L H N H Y T Q K 1380
 AGCCTCTCCCTGTCTCCGGTAAA (SEQ ID NO:18)
 1381 S L S L S P G K (SEQ ID NO:20) 1416

The complete sequence of a humanized 3D6 light chain gene with introns (located between MluI and BamHI sites, as in pVk-Hu3D6) is shown below (SEQ ID NO:15). The nucleotide number indicates its position in pVk-Hu3D6. The Vk and Ck exons are translated in single letter code; the dot indicates the translation termination codon. The mature light chain starts at the double-underlined aspartic acid (D). The intron sequence is in italics. The polyA signal is underlined. The expressed light chain corresponds to SEQ ID NO:11 when mature.

619 ACGCGTCCACCATGATGAGTCCTGCCAGTTCTGTAGTGCCTGGATTGGAAACCAACGGTGATGTTGTG
 M M S P A Q F L F L L V L W I R E T N G D = V V
 699 ATGACCCCAGTCTCCACTCTCCTGCCAGTTACCTCTGGACAACCAGCCTCCATCTCTGCCAGTCAGAGCCTCTT
 5 M T Q S P L S L P V T L G Q P A S I S C K S S Q S L L
 779 AGATAGTGTGGAAAGACATATTGAAATTGGTGTGCCAACAGGCCAGGGCCAGTCTCCAAGACGCCAATCTATCTGGTGT
 D S D G K T Y L W L Q Q R P G Q S P R R L I Y L V
 859 CTAAACTGGACTCTGGAGTCCCTGACAGGTTCTGGCAGTGGATCAGGGACAGATTACTGAAAATCAGCAGAGTC
 S K L D S . G V P D R F S G S G S G T D F T L K I S R V
 10 939 GAGGCTGAGGGATGTGGAGTTATTATTGCTGGCAAGGTACACATTTCCTCCGACGTTGGTGGAGGCCACAGGTGGA
 E A E D V G V Y Y C W Q G T H F P R T F G G G T K V E
 1019 AATCAAACGTAAGTGCACCTTCCTCTAGAAATTCTAAACTCTGAGGGGTCGGATGACGTGGCCATTCTTCCTAAAG
 I K R
 15 1099 *CATTCAGTTTACTGCAAGGTCAAGAAAAGCATGCAAAGCCCTCAGAAATGGCTGCAAAGACCTCCAAACAAAACAAATTAGAA*
 1179 *CTTTATTAAGGAATAGGGGAAGCTAGGAAGAACTCAAAACATCAAGATTAAATACGCTTCTGGTCTCTGCTAT*
 1259 *AATTATCTGGATAAACGATGCTGTTCTGCTGCCCTAACATGCCCTGTGATTATGCCAAACACACACCCAGGGC*
 1339 *AGAACCTTGTACTTAAACACCATCCCTGTTGCTTCCCTCAGGAACGTGGCTGCACATCTGCTTCACTTCCG*
 T V A A P S V F I F P
 20 1419 CCATCTGATGAGCAGTTGAAATCTGAACTGCCCTGTGTGCTGCTGAATAACTCTATCCCAGAGAGGCCAAGT
 P S D E Q L K S G T A S V V C L L N N F Y P R E A K V
 1499 ACAGTGGAAAGGTGGATAACGCCCTCAATCGGGTAACTCCAGGAGAGTGTCAACAGCAGGACAGCAAGGACGCC
 Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T
 1579 ACAGCCTCAGCAGCACCCCTGACGCCAGAACAGACTACGGAGAAACACAAAGTCTACGCCCTGCCAGTCACCCATCAG
 Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q
 25 1659 GGCCTGAGCTCGCCCGTCACAAAGCTTCACACAGGGAGACTGTTAGAGGGAGAGTGCCTCCCCACCTGCTCCTCAGTC
 G L S S P V T K S F N R G E C •
 1739 CAGCCTGACCCCCCTCCATCCTTGGCTCTGACCCCTTTCCACAGGGACCTACCCATTGCGGTCTCCAGCTCAT
 1819 *CTTTCACTTCACCCCCCTCTCTCTCTGGCTTAATTATGCTAATGTTGGAGGAGAAATGAAATAATAAA* GTGAATCTTT
 1899 GCACCTGTGGTTCTCTCTCTCTGGCTTAATTATGTTACCAACTACTCAATTCTCTTATAAGGA
 30 1979 CTAAATATGAGTCATCTCTAACGGCATAACCCATCTGCTCACAGTCCCCTGGCCATGGTAGAGAGACTTGCTTCTGCA
 2059 AGACAGTCCCTCAACCCACAAGCCTCTGCTCACAGTCCCCTGGCCATGGTAGAGAGACTTGCTTCTGCA
 2139 TTCCCCCTCTCAGCAAGCCCTCATAGCTTTTAAGGGTGACAGGTCTTACAGTCATATCCTTGATTCATACTCCCT
 2219 GAGAATCAACCAAAGCAAATTCTCAAAGAAGAAACCTGCTATAAGAGAAATCATTCAATTGAACTGATATAAAATA
 2299 CAACACAATAAAAGCAATTAAATAACAAACAATAGGGAAATGTTAAGTTCATCATGGTACTTAGACTTAATGGAATGT
 35 2379 CATGCCATTATTCACATTAAACAGGTACTGAGGGACTCTGCTGCCAAGGGCGTATTGAGTACTTCCACAACTTA
 2459 ATTAACTCACACTATCTGAGATTAAAACATTCAATTAAATGTTGCAAAGGTTCTATAAGCTGAGAGACAAATAT
 2539 ATTCTATAACTCAGCAATCCACTCTAGGATC (SEQ ID NO:15)

The complete sequence of a humanized 3D6 heavy chain gene with introns
 40 (located between MluI and BamHI sites, as in pVg1-Hu3D6) is shown below (SEQ ID
 NO:16). The nucleotide number indicates its position in pVg1-Hu3D6. The V_H and C_H
 exons are translated in single letter code; the dot indicates the translation termination
 codon. The mature heavy chain starts at the double-underlined glutamine (Q). The intron
 sequences are in italic. The polyA signal is underlined. The expressed heavy chain
 45 corresponds to SEQ ID NO:12 when mature.

619 ACGCGTCCACCATGAACTTCGGGCTCACCTGATTTCTGCTTAAAGGTGTCCAGTGTGAAGTGCACACTG
 M N F G L S L I F L V L V L K G V Q C E = V Q L
 699 GTGGAGTCTGGGGAGGGCTTAGTGCAGCCTGGAGGCTCTCTGAGGCTCTCTGTGAGGCTCTGGATTCACTTCAGTAA
 50 V E S G G G L V Q P G G S L R L S C A G S G F T F S N
 779 CTATGCCATGTCTGGGTCGCCAGGCTCTGGAAAGGGACTGGAGTGGTTGCATCCATTAGGAGTGGTGGTGTAGAA
 Y G M S W V R Q A P G K G L E W V A S I R S G G G R
 859 CCTACTATTAGACAAATGAAAGGGCGATTCAACCATCTCAGAGAGAAATGCCAAGAACAGCCTGTACCTGCAAATGAAC
 T Y Y S D N V K G R F T I S R E N A K N S L Y L Q M N
 939 AGTCTGAGAGCTGAGGACACGGCTGTCTATTAGTGTGTCAGATATGATCACTATAGTGGTAGCTCCGACTACTGGGCCA
 S L R A E D T A V Y Y C V R Y D H Y S G S S D Y W G Q
 1019 GGGCACCTGGTCACAGTCTCCTCAGGTGAGTCTCACACCTCTAGAGCTTCTGGGGCAGGCCAGGCCGACCTTGGC
 G T L V T V S S

1099 TTTGGGGCAGGGAGGGGGCTAAGGTGAGGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAGCCCAGACACTGG
 1179 ACGCTGAACCTCGCGACAGTAAAGAACCCAGGGCCCTCGCCCTGGGCCAGCTGTCCCACACCGCGGTACATG
 1259 GCACCACCTCTTCAGCCTCCACCAAGGGCCATCGGTCTTCCCCCTGGCACCCCTCTCAAGAGCACCTCTGGGGC
 A S T K G P S V F P L A P S S K S T S G G
 5 1339 ACAGCGCCCTGGCTGCGCTGGTAAGGACTACTCCCCGAACCGGTGACGGTGTGGAACCTCAGGCGCCCTGACCG
 T A A L G C L V K D Y F P E P V T V S W N S G A L T S
 1419 CGCGTGCACACCTTCCCGCTGTCTCAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTACCGTGCCCTCAGCA
 G V H T F P A V L Q S S G L Y S L S S V V T V P S S
 1499 GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGGTGA
 10 G S L G T Q T Y I C N V N H K P S N T K V D K K V
 1579 CCAGCACAGGGAGGGAGGGTGTCTGCTGAAGCCAGGCTCAGCGCTCCGCTGGACGCATCCCGCTATGCAGCCCCAG
 1659 TCCAGGGCAGCAAGGCAGGGCCCGTCTGCCTCTTCACCCGGAGGCTCTGCCCGCCCCACTCATGCTCAGGGAGGGTC
 1739 TTCTGGCTTTTCCCAGGCTCTGGGAGGGCACAGGTAGGTGCCCCCTAACCAGGCCCTGCACACAAAGGGGAGGGTC
 1819 TGGGCTCACACCTGCCAAGGAGCCATATCCGGAGGCCCTGCCCCCTGACCTAAGGCCACCCAAAGGCCAAACTCTCCAC
 15 1899 TCCCTCACCTCGGACACCTTCTCTCCAGATTCCAGTAACCTCCAACTCTCTCTCGAGAGGCCAAATCTGTGAC
 E P K S C D
 1979 AAAACTCACACATGCCAACCGTGCCAGGTAAAGCCAGGCCAGGCCTGCCAGCTCAAGGCGGACAGGTGCCCTAG
 K T H T C P P C P
 2059 AGTAGCCTGCATCCAGGGACAGGCCCGAGCCGGTGCTGACACGTCCACCTCCATCTCTCTCAGCACCTGAACCTCTG
 A P E L L
 2139 GGGGGACCGTCAGTCTCCTCTCCCCAAAACCCAAGGACACCCCATGATCTCCGGACCCCTGAGGTACATGCGT
 G G P S V F L F P P K P K D T L M I S R T P E V T C V
 2219 GGTGGTGGACGCTGAGGCCACGAAGACCTGAGGTCAAGTCACTGGTACGTGGACGGCTGGAGGTGATAATGCCAAGA
 V V D V S H E D P E V K F N W Y V D G V E V H N A K
 25 2299 CAAAGCCGGGGAGGAGCAGTACAACACGACCGTACCGTGGTCAGCGTCTCACCCTGACCCAGGACTGGCTGA
 T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N
 2379 GGCAAGGAGTACAAGTGCAGGTCTCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCAAAGCCAAGGTG
 G K E Y K C K V S N K A L P A P I E K T I S K A K
 2459 GACCCGTGGGGTCCGAGGGCCACATGGACAGGGCCGGCTGGCCACCCCTGAGGTGACACCTCTGCCCCATCCGGATG
 30 2539 TCTGTCCCTACAGGGCAGCCCCAGAGAACACAGGTGTACACCTCTGCCCCATCCGGATGACCTGACCAAGAAC
 G Q P R E P Q V Y T L P P S R D E L T K N Q V
 2619 CAGCCTGACCTGCCGTCAAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGAGAAGCAATGGCAGCCGAGAAC
 S L T C L V K G F Y P S D I A V E W E S N G Q P E N
 2699 ACTACAAGACCACGCCCTCCGTGCTGGACTCCGACGGCTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG
 35 N Y K T T P P V L D S D G S F F L Y S K L T V D K S R
 2779 TGGCAGCAGGGGAACGTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACACTACACGAGAAGAGCCT
 W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L
 2859 GTCTCCGGTAAATGAGTGCAGGGCCGGCAAGCCCCCGCTCCCGGGCTCTCGCGGTGACAGGGATGCTGGCACGT
 S P G K •
 40 2939 ACCCCCTGTACATACTTCCCGGGGCCAGCATGGAAATAA_GCACCCAGCGCTGCCCTGGGCCCTGCGAGACTGTGAT
 3019 GGTCTTTCCACGGTCAGGCCAGTCTGAGGCTGAGTGGCATGAGGGAGGAGAGCGGGCTCCACTGTCCCCACACTG
 3099 GCCCAGGCTGTGAGGTGCTGGCCCTGGCCCTAGGGCTCAGCCAGGGCTGCCCTCGGCAGGGGGATTTGC
 3179 CAGCGTGGCTCTCCAGCACCTGGCTGGCCCTGGCCAGGGCACAGACACACA
 3259 GCCCCCTGCTGTAGGAGACTGTCTGTGAGGCCCTGCTCCCTGGCCCTGGCCAGGGGGATGGAGGCTGGGGCATGCTTA
 45 3339 GTCCATGTGCGTAGGGCCCTCCCATCACCGACTAACCCCTGCGACTAACCCCTGGCTGCCCTGGCCAGGGCTCG
 3419 ACCCGCATGGGACACAACCGACTCCGGGACATGCACTCTGGGCCCTGTGGAGGGACTGGTGCAGATGCCAACACACA
 3499 CACTCAGCCCCAGACCGTTCAACAAACCCGACTGAGGTGGCCGGCACACGGCCACACACACGTGACGCC
 3579 CACACACGGAGCCTACCCGGGCAACTGCACAGCACCCAGACAGAGCAAGGTCTCGCACACGTGAACACTCTCGGA
 3659 CACAGGGCCCCACGGCCCCACGGCCACCTCAAGGCCACGAGGCTCTGGCAGCTTCTCCACATGCTGACCTGCTCAG
 50 3739 ACAAAACCCAGCCCCCTCTCACAAAGGTGCCCTGCAAGGCCACACACACAGGGATCACACACCACGTCA
 3819 TGCCCTGGCCCACTCCAGTGCAGGGCTTCCCTGCAAGGATTC (SEQ ID NO:16)

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably from immortalized B-cells. Suitable source cells for the polynucleotide sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources well-known in the art.

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and

manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework 5 regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

Alternatively, polypeptide fragments comprising only a portion of the primary 10 antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). These polypeptide fragments may be produced by proteolytic cleavage of intact antibodies by methods well known in the art, or by inserting stop codons at the desired locations in vectors using site-directed mutagenesis, such as after CH1 to produce Fab fragments or after the hinge 15 region to produce F(ab')₂ fragments. Single chain antibodies may be produced by joining VL and VH with a DNA linker.

As stated previously, the polynucleotides will be expressed in hosts after the 20 sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences.

E. coli is a prokaryotic host useful particularly for cloning the polynucleotides of 25 the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell 30 (e.g., an origin of replication). In addition, any of a number of well-known promoters may be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and

have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. *Saccharomyces* is a preferred host, with suitable vectors having expression control sequences, such as 5 promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention. Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact 10 immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, Syrian Hamster Ovary cell lines, HeLa cells, preferably myeloma cell lines, transformed B-cells, human embryonic kidney cell lines, or hybridomas. Expression vectors for these cells can include expression control sequences, such as an 15 origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, cytomegalovirus and the like.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy 20 and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Once expressed, the antibodies can be purified according to standard procedures, 25 including ammonium sulfate precipitation, ion exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel electrophoresis, and the like. Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. 30 Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or prophylactically, as directed herein.

The antibodies (including immunologically reactive fragments) are administered to a subject at risk for or exhibiting A β -related symptoms or pathology such as clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical amyloid angiopathy, using standard administration techniques, preferably peripherally (*i.e.* not by administration into the central nervous system) by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. Although the antibodies may be administered directly into the ventricular system, spinal fluid, or brain parenchyma, and techniques for addressing these locations are well known in the art, it is not necessary to utilize these more difficult procedures. The antibodies of the invention are effective when administered by the more simple techniques that rely on the peripheral circulation system. The advantages of the present invention include the ability of the antibody to exert its beneficial effects even though not provided directly to the central nervous system itself. Indeed, it has been demonstrated that the amount of antibody that crosses the blood-brain barrier is $\leq 0.1\%$ of plasma levels.

The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners.

The concentration of the humanized antibody in formulations may range from as low as about 0.1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, and so forth, in accordance with the particular mode of administration selected. Thus, a pharmaceutical composition for injection could be made up to contain in 1 mL of phosphate buffered saline from 1 to 100 mg of the humanized antibody of the present invention. The formulation could be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250 mL of fluid, such as sterile Ringer's solution, and 1-100 mg per mL, or more in antibody concentration. Therapeutic agents of the invention can be frozen or lyophilized for storage and

reconstituted in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to 5 balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 4 and 8 is tolerated.

Although the foregoing methods appear the most convenient and most appropriate for administration of proteins such as humanized antibodies, by suitable adaptation, other techniques for administration, such as transdermal administration and oral administration 10 may be employed provided proper formulation is designed. In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen. In summary, formulations are available for administering the antibodies of the invention and are well-known in the art and may be chosen from a variety of options. 15 Typical dosage levels can be optimized using standard clinical techniques and will be dependent on the mode of administration and the condition of the patient.

The following examples are intended to illustrate but not to limit the invention. Because the examples here describe experiments conducted in murine systems, the use of 20 murine monoclonal antibodies is satisfactory. However, in the treatment methods of the invention intended for human use, humanized forms of the antibodies with the immunospecificity corresponding to that of antibody 3D6 are preferred.

Example 1

Synthesis of Humanized Antibody 3D6

25 Cells and antibodies. Mouse myeloma cell line Sp2/0 was obtained from ATCC (Manassas, VA) and maintained in DME medium containing 10% FBS (Cat # SH30071.03, HyClone, Logan, UT) in a 37°C CO₂ incubator. Mouse 3D6 hybridoma cells were first grown in RPMI-1640 medium containing 10% FBS (HyClone), 10 mM HEPES, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 25 30 30 µg/ml gentamicin, and then expanded in serum-free media (Hybridoma SFM, Cat # 12045-076, Life Technologies, Rockville, MD) containing 2% low Ig FBS (Cat # 30151.03, HyClone) to a 1.5 liter volume in roller bottles. Mouse monoclonal antibody

3D6 (Mu3D6) was purified from the culture supernatant by affinity chromatography using a protein-G Sepharose column. Biotinylated Mu3D6 was prepared using EZ-Link Sulfo-NHS-LC-LC-Biotin (Cat # 21338ZZ, Pierce, Rockford, IL).

Cloning of variable region cDNAs. Total RNA was extracted from approximately 5 10^7 hybridoma cells using TRIzol reagent (Cat. # 15596-026 Life Technologies) and poly(A)⁺ RNA was isolated with the PolyATract mRNA Isolation System (Cat. # Z25310, Promega, Madison, WI) according to the suppliers' protocols. Double-stranded cDNA was synthesized using the SMARTTMRACE cDNA Amplification Kit (Cat. # K1811-1, Clontech, Palo Alto, CA) following the supplier's protocol. The variable region cDNAs 10 for the light and heavy chains were amplified by polymerase chain reaction (PCR) using 3' primers that anneal respectively to the mouse kappa and gamma chain constant regions, and a 5' universal primer provided in the SMARTTMRACE cDNA Amplification Kit. For VL PCR, the 3' primer has the sequence:

15 5' -TATAGAGCTCAAGCTTGGATGGTGGAAAGATGGATAACAGTTGGTGC-3'
[SEQ ID NO:13]

with residues 17- 46 hybridizing to the mouse Ck region. For VH PCR, the 3' primers have the degenerate sequences:

20

A G T
5' -TATAGAGCTCAAGCTTCCAGTGGATAGACCGATGGGGCTGTCGTTGGC-3'
T
[SEQ ID NO:14]

25

with residues 17 - 50 hybridizing to mouse gamma chain CH1. The VL and VH cDNAs were subcloned into pCR4Blunt-TOPO vector (Cat. # 45-0031, Invitrogen, Carlsbad, CA) for sequence determination. DNA sequencing was carried out by PCR cycle sequencing reactions with fluorescent dideoxy chain terminators (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The sequencing reactions were 30 analyzed on a Model 377 DNA Sequencer (Applied Biosystems).

Construction of humanized 3D6 (Hu3D6) variable regions. Humanization of the mouse antibody V regions was carried out as outlined by Queen et al., (1989), *op. cit.* The human V region framework used as acceptor for Mu3D6 CDRs was chosen based on

sequence homology. The computer programs ABMOD and ENCAD [Levitt, M., J. Mol. Biol. 168:595-620 (1983)] were used to construct a molecular model of the variable regions. Amino acids in the humanized V regions that were predicted to have contact with CDRs were substituted with the corresponding residues of Mu3D6. This was done at residues 49, 73, and 98 in the heavy chain and at residue 41 in the light chain. The amino acids in the humanized V region that were found to be rare in the same V-region subgroup were changed to the consensus amino acids to eliminate potential immunogenicity. This was done at residues 6 and 91 in the heavy chain.

The light and heavy chain variable region genes were constructed and amplified using eight overlapping synthetic oligonucleotides ranging in length from approximately 65 to 80 bases [He, X. Y., et al., J. Immunol. 160: 029-1035 (1998)]. The oligonucleotides were annealed pairwise and extended with the Klenow fragment of DNA polymerase I, yielding four double-stranded fragments. The resulting fragments were denatured, annealed pairwise, and extended with Klenow, yielding two fragments. These fragments were denatured, annealed pairwise, and extended once again, yielding a full-length gene. The resulting product was amplified by PCR using the Expand High Fidelity PCR System (Cat. # 1 732 650, Roche Molecular Biochemicals, Indianapolis, IN). The PCR-amplified fragments were gel-purified and cloned into pCR4Blunt-TOPO vector. After sequence confirmation, the VL and VH genes were digested with *Mlu*I and *Xba*I, gel-purified, and subcloned respectively into vectors for expression of light and heavy chains to make pV_k-Hu3D6 and pV_{g1}-Hu3D6 [Co, M. S., et al., J. Immunol. 148:1149-1154 (1992)]. The mature humanized 3D6 antibody expressed from these plasmids has the light chain of SEQ ID NO:11 and the heavy chain of SEQ ID NO:12.

Stable transfection. Stable transfection into mouse myeloma cell line Sp2/0 was accomplished by electroporation using a Gene Pulser apparatus (BioRad, Hercules, CA) at 360 V and 25 µF as described (Co, et al., 1992, *op. cit.*). Before transfection, pV_k-Hu3D6 and pV_{g1}-Hu3D6 plasmid DNAs were linearized using *Fsp*I and *Bst*Z171, respectively. Approximately 10⁷ Sp2/0 cells were transfected with 20 µg of pV_k-Hu3D6 and 40 µg of pV_{g1}-Hu3D6. The transfected cells were suspended in DME medium containing 10% FBS and plated into several 96-well plates. After 48 hr, selection media (DME medium containing 10% FBS, HT media supplement, 0.3 mg/ml xanthine and 1 µg/ml mycophenolic acid) was applied. Approximately 10 days after the initiation of the

selection, culture supernatants were assayed for antibody production by ELISA as shown below. High yielding clones were expanded in DME medium containing 10% FBS and further analyzed for antibody expression. Selected clones were then adapted to growth in Hybridoma SFM.

5 Measurement of antibody expression by ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc, Naperville, IL) were coated with 100 µl of 1 µg/ml goat anti-human IgG, Fc γ fragment specific, polyclonal antibodies (Cat # 109-005-098, Jackson ImmunoResearch, West Grove, PA) in 0.2 M sodium carbonate-bicarbonate buffer (pH 9.4) overnight at 4°C. After washing with Washing Buffer (PBS containing 0.1% Tween 20), wells were blocked with 400 µl of Superblock Blocking Buffer (Cat # 37535, Pierce) for 30 min and then washed with Washing Buffer. Samples containing Hu3D6 were appropriately diluted in ELISA Buffer (PBS containing 1% BSA and 0.1% Tween 20) and applied to ELISA plates (100 µl per well). As a standard, humanized anti-CD33 IgG1 monoclonal antibody HuM195 (Co, *et al.*, 1992, *op. cit.*) was used. The ELISA plate was incubated for 2 hr at room temperature and the wells were washed with Washing Buffer. Then, 100 µl of 1/1,000-diluted HRP-conjugated goat anti-human kappa polyclonal antibodies (Cat # 1050-05, Southern Biotechnology, Birmingham, AL) in ELISA Buffer was applied to each well. After incubating for 1 hr at room temperature and washing with Washing Buffer, 100 µl of ABTS substrate (Cat #s 20 507602 and 506502, Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. Color development was stopped by adding 100 µl of 2% oxalic acid per well. Absorbance was read at 415 nm using an OPTImax microplate reader (Molecular Devices, Menlo Park, CA).

25 Purification of Hu3D6. One of the high Hu3D6-expressing Sp2/0 stable transfecants (clone #40) was adapted to growth in Hybridoma SFM and expanded to 2 liters in roller bottles. Spent culture supernatant was harvested when cell viability reached 10% or below and loaded onto a protein-A Sepharose column. The column was washed with PBS before the antibody was eluted with 0.1 M glycine-HCl (pH 2.5), 0.1 M NaCl. The eluted protein was dialyzed against 3 changes of 2 liters of PBS and filtered through a 0.2 µm filter prior to storage at 4°C. Antibody concentration was determined by measuring absorbance at 280 nm (1 mg/ml = 1.4 A₂₈₀). SDS-PAGE in Tris-glycine buffer was performed according to standard procedures on a 4-20% gradient gel (Cat #

EC6025, Novex, San Diego, CA). Purified humanized 3D6 antibody is reduced and run on an SDS-PAGE gel. The whole antibody shows two bands of approximate molecular weights 25 kDa and 50 kDa. These results are consistent with the molecular weights of the light chain and heavy chain, or with the molecular weight of the chain(s) comprising a fragment, calculated from their amino acid compositions.

Example 2

In vitro binding properties of humanized 3D6 antibody

The binding efficacy of humanized 3D6 antibody, synthesized and purified as described above, was compared with the mouse 3D6 antibody using biotinylated mouse 3D6 antibody in a comparative ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc) were coated with 100 µl of β-amyloid peptide (1-42) in 0.2 M sodium carbonate/bicarbonate buffer (pH 9.4) (0.3 µg/mL) overnight at 4°C.

After washing the wells with phosphate buffered saline (PBS) containing 0.1% Tween 20 (Washing Buffer) using an ELISA plate washer, the wells were blocked by adding 300 µL of SuperBlock reagent (Pierce) per well. After 30 minutes of blocking, the wells were washed with Washing Buffer and excess liquid was removed.

A mixture of biotinylated Mu3D6 (0.2 µg/ml final concentration) and competitor antibody (Mu3D6 or Hu3D6; starting at 300 µg/ml final concentration and serial 3-fold dilutions) in ELISA Buffer were added in triplicate in a final volume of 100 µl per well. As a no-competitor control, 100 µl of 0.2 µg/ml biotinylated Mu3D6 was added. As a background control, 100 µl of ELISA Buffer was added. The ELISA plate was incubated at room temperature for 90 min. After washing the wells with Washing Buffer, 100 µl of 1 µg/ml HRP-conjugated streptavidin (Cat # 21124, Pierce) was added to each well. The plate was incubated at room temperature for 30 min and washed with Washing Buffer. For color development, 100 µl/well of ABTS Peroxidase Substrate (Kirkegaard & Perry Laboratories) was added. Color development was stopped by adding 100 µl/well of 2% oxalic acid. Absorbance was read at 415 nm. The absorbances were plotted against the log of the competitor concentration, curves were fit to the data points (using Prism) and the IC₅₀ was determined for each antibody using methods well-known in the art.

The mean IC₅₀ for mouse 3D6 was 2.7 µg/mL (three separate experiments, standard deviation = 0.6 µg/mL) and for humanized 3D6 was 3.3 µg/mL (three separate

experiments, standard deviation = 0.8 µg/mL). A second set of three experiments was carried out, essentially as described above, and the mean IC₅₀ for mouse 3D6 was determined to be 3.97 µg/mL (SD = 0.15 µg/mL) and for humanized 3D6, the IC₅₀ was determined to be 3.97 µg/mL (SD = 0.20 µg/mL). On the basis of these results, we
5 conclude that humanized 3D6 has binding properties that are very similar to those of the mouse antibody 3D6. Therefore, we expect that humanized 3D6 has very similar *in vitro* and *in vivo* activities compared with mouse 3D6 and will exhibit in humans the same effects demonstrated with mouse 3D6 in mice.

10

Example 3

In vitro binding properties of mouse and humanized antibodies 3D6

Antibody affinity (KD = Kd / Ka) was determined using a BIACore biosensor 2000 and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-mouse or anti-human IgG) was coupled via free amine groups to carboxyl groups on flow
15 cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG was coupled to flow cell 1 as a background control. Monoclonal antibodies were captured to yield 300 resonance units (RU). Amyloid-beta 1-40 or 1-42 (Biosource International, Inc.) was then flowed over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the
20 chip, bound anti-A β antibody was eluted from the chip using a wash with glycine-HCl (pH 2). A control injection containing no amyloid-beta served as a control for baseline subtraction. Sensorgrams demonstrating association and dissociation phases were analyzed to determine Kd and Ka. The affinity (KD) of mouse antibody 3D6 for A β 1-42 was determined to be 2.4 nM, and the affinity of humanized 3D6, prepared essentially as
25 described in Example 1, was determined to be 2.3 nM.

Example 4

Epitope mapping of mouse and humanized 3D6

The BIACore is an automated biosensor system for measuring molecular
30 interactions [Karlsson R., *et al. J. Immunol. Methods* 145:229-240 (1991)]. The advantage of the BIACore over other binding assays is that binding of the antigen can be measured without having to label or immobilize the antigen (i.e. the antigen maintains a

more native conformation). The BIACore methodology was used to assess the binding of various amyloid-beta peptide fragments to either mouse 3D6 or humanized 3D6 (prepared substantially as described in Example 1). All dilutions were made with HEPES buffered saline containing Tween 20. A single concentration of a variety of fragments of human
5 A β or mouse A β 1-40 (BioSource International) was used. Human amyloid beta fragments 1-10 and 1-20 bound to mouse 3D6 and to humanized 3D6, while human A β fragments 10-20 and 16-25 did not bind to either antibody. Neither mouse 3D6 nor humanized 3D6 bound mouse A β 1-40. Using this methodology, the binding epitope for both mouse and humanized 3D6 appears to be between amino acids 1 and 10 of human
10 A β .

Example 5

Effects of administration of 3D6

Unless otherwise stated, all studies used APP^{V717F} (PDAPP) transgenic mice, and all injections were i.p. In general, a control group of mice received injections of saline.
15 Six weeks of weekly injection of 360 μ g of 3D6 in old, hemizygous mice (24 month) lowered hippocampal insoluble A β_{total} by 10% and A β 1-42 by 1% (N.S., not statistically significant) in 9 animals per control group and 10 animals per antibody group. In the cortex, mean insoluble A β_{total} was lower by 33% and A β 1-42 by 47% ($p<0.05$), while insoluble A β 1-40 increased by 100%.

20 In hemizygous, 4 month old mice, administration of 360 μ g of 3D6 per animal: 1) raised average plasma A β 1-40 and A β 1-42 levels approximately 6-fold and 9-fold, respectively, by 24 hours after administration; and 2) had no significant effect on soluble A β 1-40 in the cortex after 24 hours compared with saline control (5 animals per group). In another study with hemizygous, 3 month old mice, administration of 360 μ g of 3D6 per
25 animal raised average plasma A β 1-42 levels approximately 8-fold by 24 hours after administration.

Administration of 360 μ g of 3D6 per animal (5 animals per group, saline control): raised average plasma A β 1-40 and A β 1-42 levels approximately 92-fold and 32-fold, respectively, by 24 hours after administration ($p<0.05$); lowered cortical insoluble A β 1-40 by 42% ($p<0.05$) and A β 1-42 by 27% (N.S.), but increased A β_{total} by 35% (N.S.); had no consistent or significant effect on soluble or insoluble A β 1-40, A β 1-42, or A β_{total} in

the hippocampus after 24 hours; in the cerebellum, increased soluble A β 1-42 by 80% ($p<0.001$) and A β_{total} by 68% (N.S.), but lowered soluble A β 1-40 by 6% (N.S.); and in the cerebellum, lowered insoluble A β 1-40, A β 1-42, and A β_{total} by 35% ($p<0.01$), 21% (N.S.), and 12% (N.S.), respectively.

5 In young mice, administration of 360 μg of 3D6 per animal (5 per group): 1) raised average plasma A β 1-42 levels approximately 3-fold by 24 hours after administration; and 2) in the cortex, lowered insoluble A β 1-40 about 10% and increased insoluble A β 1-42 about 12%.

Studies were conducted to assess the effects of 3D6 on formation of stable
10 A β :antibody complexes in biological fluids, plasma A β concentrations acutely after administration, cognitive performance after acute or chronic administration, and guanidine-extracted and immunohistochemically-detected A β deposition (in brain) after chronic administration.

Mice (3 months of age) were injected with 360 μg of 3D6. Twenty-four hours
15 following antibody administration plasma was collected and proteins were resolved by gel electrophoresis under native (non-denaturing conditions) on a polyacrylamide gel. Following transfer of size fractionated proteins to a solid matrix, complexes were immunodetected with biotinylated antibody and visualized with enhanced chemiluminescence. Unlike certain other anti-A β antibodies, no complex was detected
20 with 3D6.

Young (2-3 months of age) mice were injected with 3D6. At various times following antibody administration, plasma was collected and various A β species were determined by a sandwich ELISA. Administration of 3D6 resulted in a dose-and time-dependent increase in plasma A β levels. A β_{1-40} levels increased to a greater degree than
25 A β_{1-42} levels following 3D6 administration. In an additional study, young APP^{V717F} tg mice were treated with 360 μg 3D6 and plasma A β levels were measured at 0.5, 3, 6, and 24 h following injection. 3D6 increased plasma A β levels in a time-dependent manner.

Extensive behavioral characterization of APP^{V717F} tg mice has been performed using several memory paradigms (bar-press, 8 arm-radial maze, object recognition).
30 These mice are impaired in several learning and memory tasks, and deficits in the object recognition (OR) task worsen with age. Therefore, the OR task has been used to assess

learning and memory in APP^{V717F} tg mice. Performance in the OR task is preferentially dependent on the integrity of the medial temporal lobe (perirhinal and entorhinal cortices). The OR test relies on the spontaneous tendency of rodents to preferentially explore a novel versus familiar object.

5 On the first day of testing, mice were allowed to habituate to an open field chamber for 50 minutes. The following day, mice were placed back into the open field for two 10-min trials. During trial one, mice were allowed to explore the open field in the presence of an object (e.g., marble or die). Following a 3-hr inter-trial delay, mice were placed back into the open field with the familiar object (the same object explored 10 previously during trial 1) as well as a novel object. The time spent exploring the novel object as well as the familiar object was recorded and a recognition index (the ratio of time spent exploring the novel object x 100/ total time spent exploring both objects) was calculated for each mouse. Administration of 360 µg of 3D6 per animal 24 hours prior to the habituation session in 11-12 month old APP^{V717F} tg mice improved OR performance 15 in 2 of 8 mice tested ($p<0.05$).

Homozygous tg mice (5-6 months old) were administered weekly injections of PBS and 72, 217, and 360 µg of a non-specific IgG or 3D6 ($n = 19-30$) for 5 months. At 20 necropsy, the brains were removed and processed for Aβ ELISA assays and immunohistochemical analysis of parenchymal Aβ burden. Cortical and hippocampal tissues were homogenized in PBS. PBS-insoluble Aβ was subsequently extracted from the pellets by homogenization in 5.5 M guanidine-HCl. Following homogenization, the samples were nutated for at least 24 h prior to centrifugation and collection of the 25 guanidine extract. PBS-soluble and guanidine-extracted tissue preparations were stored at -80°C for subsequent Aβ ELISA determinations. Immunohistochemical (IHC) analysis of parenchymal Aβ burden was carried out as follows. Eight (8) µm paraffin embedded paraformaldehyde fixed tissues were labeled with rabbit polyclonal anti-Aβ antibody (against Aβ 15-30) and followed by anti-rabbit IgG fluorescent detection. Eight (8) sections of brain (7 IHC, 1 control) were examined from each animal. Treatment with 30 3D6 (360 µg) markedly and significantly reduced cortical guanidine-extracted Aβ1-42 (by ELISA) and cortical and hippocampal Aβ plaque burden (by IHC), but no effect was observed at lower 3D6 doses. Although no effect on guanidine extracted Aβ1-42 was

observed at lower 3D6 doses, these doses significantly reduced cortical and hippocampal A β plaque burden (by IHC).

Radiolabeled (15 μ Ci/mouse, 0.5 mg/mouse) 3D6 was administered to ICR (non-transgenic) mice in order to evaluate kinetics and brain distribution of the antibody after 5 administration by the intravenous route. Plasma kinetics for 3D6 immunoreactivity demonstrated a half-life of elimination of approximately 5 days. TCA-precipitable radioactivity was greater than 95% of the total plasma counts throughout the study, and declined in the plasma compartment with a terminal half-life of 3-4 days. The observation that plasma radioactivity remained predominantly TCA-precipitable 10 throughout the study suggests that the radiolabeled antibody was not significantly proteolytically degraded, nor was the 125-I label cleaved from the antibody over the time course studied. The shapes of the concentration versus time profiles as measured by ELISA and radioactivity were generally similar, with some differences in the terminal phases. There was no apparent accumulation of radiolabel in any tissue, including brain. 15 Distribution of radioactivity to the brain was minimal. The amount of radioactivity associated with the brain samples in this experiment cannot be clearly distinguished from contamination by the blood compartment during tissue processing or from antibody associated with endothelial cells in the brain vasculature.

Nine month old, hemizygous mice received PBS, a non-specific IgG, or 3D6 (500 20 μ g/week) by weekly injection for six months (PBS, 11 animals; IgG, 13 animals; and 3D6, 14 animals). Weak, but statistically significant, A β lowering in the cortex (compared to IgG) and hippocampus (compared to IgG or combined PBS/IgG controls) 25 was seen. Immunohistochemical (IHC) analysis showed strong reductions in A β plaque burden in the cortex and hippocampus of 3D6-treated mice (94% and 85% reductions, respectively, versus PBS control; p<0.05, and p<0.01, respectively).

Example 6

Administration of humanized 3D6

A preparation of an anti-A β antibody comprising a light chain having the amino 30 acid sequence of SEQ ID NO:11 and a heavy chain having the amino acid sequence of SEQ ID NO:12 (a humanized 3D6) was administered as a single intravenous bolus injection to two groups of 12 male marmosets at doses of 1 and 10 mg/kg.

Concentrations of immunoreactive anti-A β antibody declined with a half-life of elimination of approximately 4 days. C_{max} and AUC parameters increased proportionally between the 1 and 10 mg/kg dose levels. The administration of humanized 3D6 to marmosets resulted in 18 or 29-fold increase in plasma A β ₁₋₄₀ immunoreactivity after 8 hours, compared with predose concentrations in the 1 and 10 mg/kg dose groups, respectively. Animals at both dose levels had concentrations of A β ₁₋₄₀ immunoreactivity above baseline levels up to 2 weeks after antibody administration. Kinetic analysis of concentrations of A β ₁₋₄₀ immunoreactivity showed that the half-life of elimination of A β ₁₋₄₀ immunoreactivity was comparable to that of the antibody (~4 days). The pharmacokinetics of humanized 3D6 were also evaluated in male *cynomolgus* monkeys after a single intravenous administration of 1 mg/kg. Analysis of immunoreactivity showed that humanized 3D6 was eliminated from the plasma with a half-life of approximately 11-12 days.

We claim:

1. Humanized 3D6 antibody.
2. A humanized antibody, or fragment thereof, comprising a humanized light chain comprising three light chain complementarity determining regions (CDRs) from the mouse monoclonal antibody 3D6 and a light chain variable region framework sequence from a human immunoglobulin light chain; and a humanized heavy chain comprising three heavy chain CDRs from the mouse monoclonal antibody 3D6 and a heavy chain variable region framework sequence from a human immunoglobulin heavy chain; wherein the light chain CDRs have the following amino acid sequences:

10 light chain CDR1:

1	5	10	15												
Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asp	Ser	Asp	Gly	Lys	Thr	Tyr	Leu	Asn
(SEQ ID NO:1)															

15 light chain CDR2:

1	5					
Leu	Val	Ser	Lys	Leu	Asp	Ser
(SEQ ID NO:2)						

light chain CDR3:

1								
Trp	Gln	Gly	Thr	His	Phe	Pro	Arg	Thr
(SEQ ID NO:3)								

and the heavy chain CDRs have the following amino acid sequences:

heavy chain CDR1:

1	5			
Asn	Tyr	Gly	Met	Ser
(SEQ ID NO:4)				

heavy chain CDR2:

1	5	10	15													
Ser	Ile	Arg	Ser	Gly	Gly	Gly	Arg	Thr	Tyr	Tyr	Ser	Asp	Asn	Val	Lys	Gly
(SEQ ID NO:5)																

and, heavy chain CDR3:

1	5	10							
Tyr	Asp	His	Tyr	Ser	Gly	Ser	Ser	Asp	Tyr
(SEQ ID NO:6)									

3. A humanized antibody or fragment thereof comprising a humanized light chain variable region having the sequence of SEQ ID NO:7 and a humanized heavy variable region having the sequence of SEQ ID NO:8.

4. The humanized antibody or fragment thereof of claim 3 having a light chain variable region of the sequence given by SEQ ID NO:9 and a heavy chain variable region given by SEQ ID NO:10.

5. The humanized antibody or fragment thereof of claim 3 having a light chain of the sequence given by SEQ ID NO:11 and a heavy chain of the sequence given by SEQ ID NO:12.

6. An antibody fragment obtainable by enzymatic cleavage of the humanized antibody of any one of claims 1 - 5.

7. An Fab or F(ab')₂ fragment of any one of the humanized antibodies of
10 claims 1 - 5.

8. The F(ab')₂ fragment of claim 7.

9. The Fab fragment of claim 7.

10. The humanized antibody or fragment of any one of claims 1 - 9, which is a single chain antibody.

15 11. The humanized antibody or fragment of any one of claims 1 - 10 that is an IgG₁ immunoglobulin isotype.

12. The humanized antibody or fragment of any one of claims 1 - 11, wherein the antibody or fragment thereof is produced in a host cell selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell,
20 and a human embryonic kidney cell.

13. A polynucleotide compound, comprising a sequence coding for the light chain or the heavy chain of the humanized antibody of any one of claims 1 - 12, or a fragment thereof.

14. A polynucleotide sequence, which when expressed in a suitable host cell, yields an antibody of any one of claims 1 – 12.

15. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 17, and a polynucleotide comprising a sequence that codes for the light chain variable region given by SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO: 11.

16. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:18, and a polynucleotide comprising a sequence that codes for the heavy chain variable region given by SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:12.

17. An expression vector for expressing the antibody of any one of claims 1 – 12 comprising the polynucleotide sequence of any one of claims 13 - 16.

18. A cell transfected with the expression vector of claim 17.

19. A cell transfected with two expression vectors of claim 17, wherein a first vector comprises the polynucleotide sequence coding for the light chain and a second vector comprises the sequence coding for the heavy chain.

20. A cell that is capable of expressing the humanized antibody or fragment of any one of claims 1 – 12.

21. The cell of any one of claims 18 – 20, wherein the cell is selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

22. A pharmaceutical composition comprising the humanized antibody or fragment of any one of claims 1 – 12, and a pharmaceutically acceptable excipient.

23. A method of treating Down's syndrome, clinical or pre-clinical Alzheimer's disease, or clinical or pre-clinical cerebral amyloid angiopathy in a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

5 24. A method to inhibit the formation of A_β plaque in the brain of a human subject, comprising administering to the human subject an effective amount of the humanized antibody or fragment of any one of claims 1 – 12.

10 25. A method to reduce A_β plaque in the brain of a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

26. The method of either of claims 24 – 25, wherein the subject is diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

15 27. The method of any one of claims 24 – 25, wherein the subject is not diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

20 28. Use of the humanized antibody or a fragment thereof according to any one of Claims 1 – 12 for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

29. Use of the humanized antibody or fragment of any one of claims 1 – 12 for the manufacture of a medicament for treating Alzheimer's disease.

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35 40 45

Pro Xaa Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
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Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

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<213> Artificial sequence

<220>

<223> humanized antibody

<400> 10

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile Arg Ser Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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<213> Artificial sequence

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<223> humanized antibody

<400> 11

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
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Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Arg Thr Phe Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 12

<211> 449

<212> PRT

<213> Artificial sequence

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<223> humanized antibody

<400> 12

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile Arg Ser Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 13

<211> 46

<212> DNA

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<223> DNA primer

<400> 13

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<210> 14

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<212> DNA

<213> Artificial sequence

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<223> DNA primer

<400> 14

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<210> 15

<211> 1953

<212> DNA

<213> Artificial sequence

<220>

<223> humanized antibody

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120

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180

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240

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<210> 16

<211> 3244

<212> DNA

<213> Artificial sequence

<220>

<223> humanized antibody

<400> 16

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<211> 717

<212> DNA

<213> Artificial sequence

<220>

<223> humanized antibody

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<210> 18

<211> 1404

<212> DNA

<213> Artificial sequence

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<223> humanized antibody

<400> 18

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<210> 19

<211> 239

<212> PRT

<213> Artificial sequence

<220>

<223> humanized antibody

<400> 19

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					20			25					30		

Val	Thr	Leu	Gly	Gln	Pro	Ala	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Gln	Ser
					35			40			45				

Leu	Leu	Asp	Ser	Asp	Gly	Lys	Thr	Tyr	Leu	Asn	Trp	Leu	Gln	Gln	Arg
					50			55			60				

Pro Gly Gln Ser Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys
115 120 125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 20

<211> 468

<212> PRT

<213> Artificial sequence

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<223> humanized antibody

<400> 20

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Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe
35 40 45

Ser Asn Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ala Ser Ile Arg Ser Gly Gly Arg Thr Tyr Tyr Ser
65 70 75 80

Asp Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
145 150 155 160

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
225 230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

Ser Pro Gly Lys
465

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 November 2002 (07.11.2002)

PCT

(10) International Publication Number
WO 02/088307 A2

(51) International Patent Classification⁷:

C12N

(21) International Application Number:

PCT/US02/11854

(22) International Filing Date:

26 April 2002 (26.04.2002)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/287,653 30 April 2001 (30.04.2001) US

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(81) Designated States (national): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/088307 A2

(54) Title: HUMANIZED ANTIBODIES

(57) Abstract: Humanized forms of mouse antibody 10D5 that retain the binding properties of mouse 10D5 are disclosed. Also disclosed are processes for making the humanized antibody, intermediates for making the humanized antibodies, including, nucleotide sequences, vectors, transformed host cells, and methods of using the humanized antibody to treat, prevent, alleviate, reverse, or otherwise ameliorate symptoms or pathology or both, that are associated with Down's syndrome or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy.

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HUMANIZED ANTIBODIES

This application claims priority of US 60/287,653, filed 2001 April 30, the entire contents of which are incorporated herein by reference.

The invention relates to humanized antibodies useful for treating and preventing 5 human diseases associated with amyloid β (A β), such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy. Mouse monoclonal antibody 10D5 was raised by immunizing mice with human A β 1-28, and has been widely used in analytical methods [*J. Neuropathol. Exper. Neurology* 51:76-83 (1992); *Nature* 359:325-327 (1992); *Neuroscience Lett.* 172:122-124 (1994); *Biochem. Biophys. Res. Commun.* 200:1598-1603 (1994); *J. Neuropathol. Exper. Neurology* 53:377-383 (1994); *Annals Neurology* 37:512-518 (1995); *Annals Neurology* 41:809-813 (1997); *J. Neuroimmunol.* 88:85-90 (1998); *J. Neuroimmunol.* 95:136-142 (1999)]. 10D5 has been shown to bind to 10 the N-terminal region of A β and has affinity of approximately 43 pM for aggregated A β .

After 10D5 was administered to a group of 8.5 to 10.5 month-old heterozygous, 15 transgenic PDAPP mice (APP^{V717F}) at a weekly intraperitoneal dose of about 10 mg/kg for six months, the mice had significantly reduced levels of A β 1-42 in brain cortex. However, the 10D5 group did not have a significant reduction of total A β in any tissue, nor of A β 1-42 in hippocampus or cerebellum [Bard, F., et al., *Nature Med.* 6:916-919 (2000); WO 00/72876 and WO 00/72880, 7 December, 2000]. It was asserted that 20 amyloid plaques in the 10D5 group also reduced in number and appearance, with some evidence of cell-associated immunoreactivity.

Another study in WO 00/72876 and WO 00/72880 reported that administration of 10D5 to older mice for six months caused a significant reduction in amyloid β plaque burden. It was asserted that the antibody gained access to the central nervous system in 25 sufficient amounts to "decorate" β -amyloid plaques. Finally, it was stated that mouse 10D5 induces phagocytosis of amyloid plaques in *in vitro* studies.

Methods for administering aggregated A β 1-42 to provoke an immunologic response and reduced amyloid deposits are described in PCT publication WO99/27944, published 10 June 1999. The description postulates that full-length aggregated A β 30 peptide would be a useful immunogen. The application also indicates that antibodies that bind to A β peptide could be used as alternate therapeutic agents. However, this appears

to be speculation since the supporting data reflect protocols that involve active immunization using, for example, A β 1-42.

WO 99/60024, published 25 November 1999, is directed to methods for amyloid removal using anti-amyloid antibodies. The mechanism, however, is stated to utilize the ability of anti-A β antibodies to bind to pre-formed amyloid deposits (i.e. plaques) and result in subsequent microglial clearance of localized plaques. This mechanism was not proved *in vivo*. This publication further states that to be effective against A β plaques, anti-A β antibodies must be delivered directly to the brain, because antibodies cannot cross the blood brain barrier.

10 Queen, *et al.* describe methods of humanizing antibodies [e.g., US Patent Nos. 5,585,089, 5,693,761, 5,693,762, 6,180,370].

15 Humanized forms of 10D5 are needed for use in humans having Down's syndrome, or pre-clinical or clinical Alzheimer's disease or cerebral amyloid angiopathy (CAA). However, it is not known whether 10D5 can be humanized so that the humanized antibody retained the binding properties of the mouse antibody.

Summary of the Invention

This invention provides humanized forms of 10D5. These humanized antibodies have binding properties (affinity and epitope location) that are approximately the same as those of the mouse 10D5 antibody. The invention includes antibodies, single chain antibodies, and fragments thereof. The invention includes antibodies wherein the CDR are those of mouse monoclonal antibody 10D5 (sequences SEQ ID NO:1 through SEQ ID NO:6) and wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody. In another aspect, this invention provides humanized antibodies and fragments thereof, wherein the variable regions have sequences comprising the CDR from mouse antibody 10D5 and specific human framework sequences (sequences SEQ ID NO:7 - SEQ ID NO:10), wherein the antibodies retain approximately the binding properties of the mouse antibody and have *in vitro* and *in vivo* properties functionally equivalent to the mouse antibody 10D5. In another aspect, this invention provides 25 humanized antibodies and fragments thereof, wherein the light chain is SEQ ID NO:11 and the heavy chain is SEQ ID NO:12.

Also part of the invention are polynucleotide sequences that encode the humanized antibodies or fragments thereof disclosed above, vectors comprising the polynucleotide sequences encoding the humanized antibodies or fragments thereof, host cells transformed with the vectors or incorporating the polynucleotides that express the humanized antibodies or fragments thereof, pharmaceutical formulations of the humanized antibodies and fragments thereof disclosed herein, and methods of making and using the same.

Such humanized antibodies and fragments thereof are useful for, among other things, treating and preventing diseases and conditions characterized by A β plaques or A β toxicity in the brain, such as Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy in humans.

The invention also includes use of a humanized antibody of the present invention for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating, preventing, or reversing Alzheimer's disease, Down's syndrome, or cerebral amyloid angiopathy, or to inhibit the formation of amyloid plaques or the effects of toxic soluble A β species in humans.

Detailed Description of the Invention

We have surprisingly found that humanized antibodies, wherein the CDRs originate from mouse monoclonal antibody 10D5 and the framework and other portions of the antibodies originate from a human germ line, bind A β 1-40 and A β 1-42 with at least the affinity with which mouse 10D5 binds A β . Thus, we have a reasonable basis for believing that humanized antibodies of this specificity, modified to reduce their immunogenicity by converting them to a humanized form, offer the opportunity to treat, both prophylactically and therapeutically, conditions in humans that are associated with formation of beta-amyloid plaques. These conditions include, as noted above, pre-clinical and clinical Alzheimer's, Down's syndrome, and pre-clinical and clinical cerebral amyloid angiopathy.

As used herein, the word "treat" includes therapeutic treatment, where a condition to be treated is already known to be present and prophylaxis - *i.e.*, prevention of, or amelioration of, the possible future onset of a condition.

- By "antibody" is meant a monoclonal antibody *per se*, or an immunologically effective fragment thereof, such as an Fab, Fab', or F(ab')₂ fragment thereof. In some contexts, herein, fragments will be mentioned specifically for emphasis; nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as the protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly.
- Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred.
- 10 Antibodies are properly cross-linked via disulfide bonds, as is well known.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 3 or more amino acids.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat, et al., "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, et al., J. Mol. Biol. 196:901-917 (1987); Chothia, et al., Nature 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). A humanized immunoglobulin does not encompass a chimeric antibody, having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Humanized antibodies have at least three potential advantages over non-human and chimeric antibodies for use in human therapy:

- 1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).
- 2) The human immune system should not recognize the framework or C region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody.
- 3) Injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected humanized antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The design of humanized immunoglobulins may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used

(acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor immunoglobulin):

- (a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position;
- (b) the position of the amino acid is immediately adjacent to one of the CDRs; or
- (c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, *et al.*, Proc. Natl. Acad. Sci. USA 86:10029-10033 (1989), and Co, *et al.*, Proc. Natl. Acad. Sci. USA 88, 2869 (1991)]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

A preferred humanized antibody is a humanized form of mouse antibody 10D5.

The CDRs of humanized 10D5 have the following amino acid sequences:

light chain CDR1:

1 5 10 15
20 Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)

light chain CDR2:

1 5
25 Lys Val Ser Asn Arg Phe Ser (SEQ ID NO:2)

light chain CDR3:

1 5
Phe Gln Gly Ser His Val Pro Leu Thr (SEQ ID NO:3)

30 heavy chain CDR1:

1 5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)

35 heavy chain CDR2:

1 5 10 15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)

40 and, heavy chain CDR3:

1 5 10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr (SEQ ID NO:6).

A preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK18 and J segment Jk4:

5	1	5	10	15
	Asp Val Xaa Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Leu Gly			
10	20	25	30	
	Xaa Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Xaa His Ser			
15	35	40	45	
	Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser			
20	50	55	60	
	Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro			
25	65	70	75	80
	Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile			
	85	90	95	
	Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Phe Gln Gly			
	100	105	110	
	Ser His Val Pro Leu Thr Phe Gly Xaa Gly Thr Lys Xaa Glu Ile Lys			
	Arg			(SEQ ID NO:7).

wherein:

- Xaa at position 3 is Val or Leu;
- 30 Xaa at position 7 is Ser or Thr;
- Xaa at position 14 is Thr or Ser;
- Xaa at position 17 is Gln, Asp, or Asn;
- Xaa at position 30 is Ile or Val;
- Xaa at position 50 is Arg or Lys;
- 35 Xaa at position 88 is Val or Leu;
- Xaa at position 105 is Gly or Ala; and
- Xaa at position 109 is Val or Leu.

A preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP-28 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity:

1 5 10 15
 Xaa Xaa Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu

20 25 30
 5 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

35 40 45
 Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

10 50 55 60
 Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Xaa

65 70 75 80
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val

15 85 90 95
 Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr

20 100 105 110
 Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr

115 120
 Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser (SEQ ID NO:8).

25 wherein:
 Xaa at position 1 is Gln or Glu;
 Xaa at position 2 is Val or Ala;
 Xaa at position 64 is Ser or Thr;
 Xaa at position 77 is Lys or Arg;
 30 Xaa at position 78 is Ser or Thr;
 Xaa at position 83 is Thr or Ser;
 Xaa at position 84 is Met, Ile, or Leu;
 Xaa at position 86 is Asn, Ser, or Thr;
 Xaa at position 87 is Met, Val, or Leu; and
 35 Xaa at position 118 is Leu or Ser.

A particularly preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segment DPK18 and J segment Jk4:

40 1 5 10 15
 Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly

20 25 30
 45 Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser

35 40 45
 Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser

	50	55	60	
	Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro			
5	65	70	75	80
	Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile			
10	85	90	95	
	Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly			
	100	105	110	
	Ser His Val Pro Leu Thr Phe Gly Gly Thr Lys Val Glu Ile Lys			
15	Arg			(SEQ ID NO: 9).

A particularly preferred heavy chain variable region of a humanized antibody of
20 the present invention has the following amino acid sequence, in which the framework
originated from human germline VH segment DP-28 and J segment JH4:

	1	5	10	15	
	Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu				
25	20	25	30		
	Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser				
30	35	40	45		
	Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu				
	50	55	60		
	Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser				
35	65	70	75	80	
	Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val				
	85	90	95		
	Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr				
40	100	105	110		
	Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr				
45	115	120			
	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser				(SEQ ID NO: 10).

A preferred light chain for a humanized antibody of the present invention has the
50 amino acid sequence:

	1	5	10	15	
	Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly				
55	20	25	30		
	Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser				

	35	40	45	
	Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser			
5	50	55	60	
	Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro			
	65	70	75	80
	Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile			
10	85	90	95	
	Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly			
	100	105	110	
	Ser His Val Pro Leu Thr Phe Gly Gly Thr Lys Val Glu Ile Lys			
15	115	120	125	
	Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu			
	130	135	140	
20	Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe			
	145	150	155	160
	Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln			
25	165	170	175	
	Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser			
	180	185	190	
30	Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu			
	195	200	205	
	Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser			
	210	215		
35	Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys			(SEQ ID NO:11).

A preferred heavy chain for a humanized antibody of the present invention has the amino acid sequence:

40	1	5	10	15
	Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu			
	20	25	30	
45	Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser			
	35	40	45	
	Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu			
50	50	55	60	
	Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser			
	65	70	75	80
	Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val			
55	85	90	95	
	Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr			
	100	105	110	
60	Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr			

115 120 125
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 130 135 140
 5 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 145 150 155 160
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 165 170 175
 10 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 180 185 190
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 195 200 205
 15 Thr Val Pro Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 210 215 220
 20 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
 225 230 235 240
 Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 245 250 255
 25 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 260 265 270
 30 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 275 280 285
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 290 295 300
 35 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 305 310 315 320
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 325 330 335
 40 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 340 345 350
 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 355 360 365
 45 Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 370 375 380
 50 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 385 390 395 400
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 405 410 415
 55 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 420 425 430
 60 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 435 440 445
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

450
Leu Ser Pro Gly Lys

(SEQ ID NO:12).

5

Other sequences are possible for the light and heavy chains for humanized 10D5. The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least one chain comprising one or more mouse complementarity determining regions functionally joined to human framework region segments.

10 In another aspect, the present invention is directed to recombinant polynucleotides encoding antibodies which, when expressed, comprise the heavy and light chain CDRs from an antibody of the present invention. Exemplary polynucleotides, which on expression code for the polypeptide chains comprising the heavy and light chain CDRs of monoclonal antibody 10D5 are given herein. Due to codon degeneracy, other 15 polynucleotide sequences can be readily substituted for those sequences. Particularly preferred polynucleotides of the present invention encode antibodies, which when expressed, comprise the CDRs of SEQ ID NO:1 – SEQ ID NO:6, or any of the variable regions of SEQ ID NO:7 – SEQ ID NO:10, or the light and heavy chains of SEQ ID NO:11 and SEQ ID NO:12.

20 The polynucleotides will typically further include an expression control polynucleotide sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic 25 hosts may also be used. Once the vector has been incorporated into the appropriate host cell line, the host cell is propagated under conditions suitable for expressing the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

30 The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), using any of a variety of well known

techniques. Joining appropriate genomic and synthetic sequences is a common method of production, but cDNA sequences may also be utilized.

Below is a cDNA sequence (SEQ ID NO:17), from which the light chain having the amino acid sequence of SEQ ID NO:19 may be expressed.

5 ATGAAGTTGCCTGTTAGGCTTGGTACTGATGTTCTGGATTCCCTGCTTCCAGCAGTGAT
 1 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 60
 M K L P V R L L V L M F W I P A S S S D -

 10 GTTGTGATGCCCAATCTCACTCTCCCTGCCTGTCACTCTTGGACAGCCAGCCTCCATC
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 120
 V V M T Q S P L S L P V T L G Q P A S I -

 15 TCTTGAGATCTAGTCAGAACATTATAACATAGTAATGGAAACACCTATTTAGAATGGTAC
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 180
 S C R S S Q N I I H S N G N T Y L E W Y -

 20 CTGCAGAAACCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTCCAACCGATTTCT
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
 L Q K P G Q S P R L L I Y K V S N R F S -

 25 GGGGTCCCAGACAGGTTCAAGTGGCAGTGGATCAGGGACAGATTCACACTCAAGATCAGC
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 300
 G V P D R F S G S G S G T D F T L K I S -

 30 AGAGTGGAGGCTGAGGATGTGGAGTTATTACTGCTTCAGGTTCACATGTTCCGCTC
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 360
 R V E A E D V G V Y Y C F Q G S H V P L -

 35 ACTTTGGCGGAGGGACCAAGGTGGAAATAAAACGAACGTGGCTGCACCATCTGTCCTC
 361 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 420
 T F G G G T K V E I K R T V A A P S V F -

 40 ATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGCGCTGCTG
 421 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
 I F P P S D E Q L K S G T A S V V C L L -

 45 ATAAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCG
 481 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
 N N F Y P R E A K V Q W K V D N A L Q S -

 50 GGTAACTCCCAGGAGAGTGTACAGAGCAGGCCAGCAAGGACAGCACCTACAGCCTCAGC
 541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
 G N S Q E S V T E Q D S K D S T Y S L S -

 55 AGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAAACACAAAGTCTACGCCCTGCGAAGTC
 601 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 660
 S T L T L S K A D Y E K H K V Y A C E V -

 60 ACCCATCAGGGCCTGAGCTGCCCGTCACAAAGAGCTAACAGGGGAGAGTGT (SEQ ID NO:17)
 661 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 714
 T H Q G L S S P V T K S F N R G E C - (SEQ ID NO:19)

55 Below is a cDNA sequence (SEQ ID NO:18), from which the heavy chain having the amino acid sequence of SEQ ID NO:20 may be expressed.

60 ATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCTGCATATGTCCTGTCCCAG
 1 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 60
 M D R L T S S F L L L I V P A Y V L S Q -

GTTACTCTGAAAGAGTCTGGCCCTGTACTAGTGAAGCCCACCGAGACCCTCACTCTGACT
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 120
 V T L K E S G P V L V K P T E T L T L T -
 5
 TGTACTTTCTCTGGTTTCACTGAGCACTCTGGTATGGGAGTGAGCTGGATTGGCAG
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 180
 C T F S G F S L S T S G M G V S W I R Q -
 10
 CCTCCAGGAAAGGCTCTGGAGTGGCTGGCACACATTTACTGGGATGATGACAAGCGCTAT
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
 P P G K A L E W L A H I Y W D D D K R Y -
 15
 AACCCATCCCTGAAGAGCCGGCTCACAACTCCAAGGATACCTCCAAAAGCCAGGTAGTC
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 300
 N P S L K S R L T I S K D T S K S Q V V -
 20
 CTCACGATGACCAATATGGACCCCTGTAGATACTGCCACATACTACTGTGTTCGAAGGCC
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 360
 L T M T N M D P V D T A T Y Y Y C V R R P -
 25
 ATTACTCCGGTACTAGTCGATGCTATGGACTACTGGGGCAAGGAACCCCTGGTACCGTC
 361 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 420
 I T P V L V D A M D Y W G Q G T L V T V -
 30
 TCCTCAGCCTCCACCAAGGGCCATCGGTCTTCCCCCTGGCACCCCTCCCAAGAGCACC
 421 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
 S S A S T K G P S V F P L A P S S K S T -
 35
 TCTGGGGGCACAGCGGCCCTGGCTGCCCTGGTAAGGACTACTTCCCCGAACCGGTGACG
 481 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
 S G G T A A L G C L V K D Y F P E P V T -
 40
 GTGTCGTTGAACTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGCTGTCTACAG
 541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
 V S W N S G A L T S G V H T F P A V L Q -
 45
 TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGCACC
 601 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 660
 S S G L Y S L S S V V T V P S S S L G T -
 50
 CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
 661 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
 Q T Y I C N V N H K P S N T K V D K K V -
 55
 GAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACCTCG
 721 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 780
 E P K S C D K T H T C P P C P A P E L L -
 60
 GGGGGACCGTCAGTCTTCCTTCCCCAAAACCCAAAGGACACCCCATGATCTCCGG
 781 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 840
 G G P S V F L F P P K P K D T L M I S R -
 65
 ACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC
 841 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 900
 T P E V T C V V V D V S H E D P E V K F -
 70
 AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAACAGCAAGCCGGAGGAGCAG
 901 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
 N W Y V D G V E V H N A K T K P R E E Q -
 75
 TACAAACAGCACGTACCGTGTGGTCAGCGTCCCTCACCGTCTGCACCAAGGACTGGCTGAAT
 961 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020
 Y N S T Y R V V S V L T V L H Q D W L N -
 80
 GGCAAGGAGTACAAGTGCAGGTCTCCAAACAAAGCCCTCCAGCCCCCATCGAGAAAACC
 1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080
 G K E Y K C K V S N K A L P A P I E K T -

ATCTCCAAAGCCAAGGGCAGCCCCGAGAACCAACAGGTGTACACCCCTGCCCATCCCGG
 1081 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1140
 I S K A K G Q P R E P Q V Y T L P P S R -
 5
 GATGAGCTGACCAAGAACCGAGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
 1141 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
 D E L T K N Q V S L T C L V K G F Y P S -
 10
 GACATCGCCGTGGAGTGGGAGAGCAATGGGAGCCGGAGAACAACTACAAGACCACGCC
 1201 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1260
 D I A V E W E S N G Q P E N N Y K T T P -
 15
 CCCGTGCTGGACTCCGACGGCTCCTCTTCTACAGCAAGCTCACCGTGAGACAGAGC
 1261 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1320
 P V L D S D G S F F L Y S K L T V D K S -
 20
 AGGTGGCAGCAGGGAAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAACAC
 1321 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1380
 R W Q Q G N V F S C S V M H E A L H N H -
 TACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAA (SEQ ID NO:18)
 1381 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1416
 Y T Q K S L S L S P G K - (SEQ ID NO:20)
 25

The complete sequence of a humanized 10D5 light chain gene with introns (located between MluI and BamHI sites, as in pV_k-Hu10D5) is shown below (SEQ ID NO:15). The nucleotide number indicates its position in pV_k-Hu10D5. The V_k and C_k exons are translated in single letter code; the dot indicates the translation termination codon. The mature light chain starts at the double-underlined aspartic acid (D). The intron sequences are in italic. The expressed light chain corresponds to SEQ ID NO:11 when mature.

619 ACGCGTCCACCATGAAGTTGCCTGTTAGGCTGTTGACTGATGTTCTGGATTCCCTGCTTCCAGCAGTGATGTTGTGATG
 M K L P V R L L V L M F W I P A S S S D = V V M
 35 699 ACCCAATCTCCACTCTCCCTGCCTGCACTCTGGACAGCCAGCCTCCATCTCTGCAGATCTAGTCAGAACATTATAACA
 T Q S P L S L P V T L Q G Q P A S I S C R S S Q N I H
 779 TAGTAATGGAAACACCTATTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCAAGGCTCCTGATCTACAAAGTTCCA
 S N G N T Y L E W Y L Q K P G Q S P R L L I Y K V S
 859 ACCGATTTCTGGGGTCCCAGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAG
 40 N R F S G V P D R F S G S G S G T D F T L K I S R V E
 939 GCTGAGGATGTGGGAGTTTAACTGCTTCAAGGTTCACATGTTCCGCTCACTTTCGGGGAGGGACCAAGGTGGAAAT
 A E D V G Y Y C F Q G S H V P L T F G G G T K V E I
 1019 AAAACGTAAGTGCACTTCTCTAACTCTAGAAATTCTAAACTCTGAGGGGGTGGATGACGTGGCCATTCTTGCCTAAAGC
 K R
 45 1099 ATTGAGTTTACTGCAAGGTCAAGAAAGCATGCAAAGCCCTCAGAATGGCTGCAAAGAGCTCCAACAAACAAATTAGAAC
 1179 TTTATTAAGGAATAGGGGGAGCTAGGAAGAAACTCAAACATCAAGATTAAATACGCTTCTTGGTCTCCCTGCTATA
 1259 ATTATCTGGGATAAGCATGCTGTTCTGCTGCTCTAACATGCCCTGTGATTATCCGAAACACACCCCAAGGGCA
 1339 GAACTTTGTTACTTAAACACCATCTGTTGCTCTTCTCAGGAACGTGGCTGCACCATCTGTCATCTCCGC
 T V A A P S V F I F P
 50 1419 CATCTGATGAGCAGTTGAAATCTGGAAC TGCTCTGTTGTGCTGCTGAATAACCTCTATCCCAGAGAGGCCAAAGTA
 P S D E Q L K S G T A S V V C L L N N F Y P R E A K V
 1499 CAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTA
 Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y
 1579 CAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGG
 S L S S T L T L S K A D Y E K H K V Y A C E V T H Q
 1659 GCCTGAGCTCGCCCCGTACAAAGAGCTTCAACAGGGAGAGTGTAGAGGGAGAACTGCCCTACCTGCTCCAGTCC
 G L S S P V T K S F N R G E C •
 1739 AGCCTGACCCCTCCCATCCTTGGCTCTGACCCCTTTCCAGGGGACCTACCCCTATTGCGGCTCTCCAGCTCATC
 1819 TTTCACCTCACCCCCCTCCTCCTGGCTTAATTGCTAATGTTGGAGGAGAATGAATAAATAA GTGAATCTTGC

1899 CACCTGTGGTTCTCTTCCCTATTTAATAATTATTATCTGTTGTTTACCAACTACTCAATTCTTATAAGGGAC
 1979 TAAATATGTAGTCATCTAACGGCGCATAACCATTATAAAAATCATCCTCATCTATCTTACCCCTATCCTCTGCAA
 2059 GACAGTCCTCCCTCAAAACCAAGGCCATGGCTTCAGTCCCCTGGGCATGGTAGGAGACTTGCTTCTGTTTT
 2139 TCCCCCTCTCAGCAAGCCCTCATGCTTTAAGGGTGACAGGCTTACAGTCATATATCCTTGATTCAATTCCG
 5 2219 AGAATCAACCAAAGCAAATTTCAAAAGAGAAACCTGCTATAAGAGAAATCATTCATGCAACATGATAAAAATAC
 2299 AACACAATAAAAGCAATTAAATAACAAACATAGGGAAATGTTAAGTCATCATGGTACTTAGACTTAATGGAATGTC
 2379 ATGCCTTATTACATTAAACAGGTAAGGGACTCTGCTGCCAAGGGCGTATTGAGTACTTTCCACAAACCTAA
 2459 TTAAATCCACACTATACTGTGAGATTAACATCATTAAAATGTTGCAAAGGTTCTATAAGCTGAGAGACAAATATA
 2539 TTCTATAACTCAGCAATCCACTTCTAGGATCC (SEQ ID NO:15)

10

The complete sequence of a humanized 10D5 heavy chain gene with introns (located between MluI and BamHI sites, as in pVg1-Hu10D5) is shown below (SEQ ID NO:16). The nucleotide number indicates its position in pVg1-Hu10D5. The V_H and C_H exons are translated in single letter code; the dot indicates the translation termination codon. The mature heavy chain starts at the double-underlined glutamine (Q). The intron sequences are in italic. The expressed heavy chain corresponds to SEQ ID NO:12 when mature.

20 619 ACGCGTCCACCATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGCTCCCTGCATATGTCCTGTCCCAGGTTACTCTG
 M D R L T S S F L L L I V P A Y V L S Q = V T L
 699 AAAGAGTCTGGCCCTGTAAGTGAAGGCCACCAGACCCCTCACTGACTTGACTTTCTCTGGGTTTCACTGAGCAC
 K E S G P V L V K P T E T L T L T C T F S G F S L S T
 779 *TTCCTGGTATGGGAGTGGATCTGTCAGCCTCCAGGAAGGCTCTGGAGTGGCTGGCACACATTACTGGGATGATG*
 S G M G V S W I R Q P P G K A L E W L A H I Y W D D
 25 859 ACAAGCGCTATAACCCATCCCTGAAGAGCCGGTCAACATCCAAGGATACCTCCAAAAGCCAGGTAGTCCTCACGATG
 D K R Y N P S L K S R L T I S K D T S K S Q V V L T M
 939 ACCAATATGGACCCCTGTAAGATACTGCCACATAACTACTGTGTCGAAGGCCATTACTCCGGTACTAGTCGATGCTATGGA
 T N M D P V D T A T Y Y C V R R P I T P V L V D A M D
 1019 CTACTGGGCAAGGAACCCCTGGTCACCGTCTCTCAGGTGAGTCCTCACAACTCTAGAGCTTCTGGGCAGGCCAGG
 Y W G Q G T L V T V S S
 1099 *CCTGACCTTGGCTTGGGGCAAGGGAGGGGCTAACGTGAGGCAGGTGGCCAGCCAGGTGCACACCCATGCCATGAG*
 1179 CCCAGACACTGGACGCTGAACCTCGCCGACAGTTAAGAACCCAGGGGCTCTGCGCCCTGGGCCAGCTGTCCCACAC
 1259 CGCGCTCACATGGCACCAACCTCTTGCAGCCTCACCAAGGCCATCGCTTCCCCCTGGCACCCCTCCAAAGAGC
 A S T K G P S V F P L A P S S K S
 35 1339 ACCTCTGGGGCACAGCGCCCTGGCTGCCCTGGTAAGGACTACTTCCCGAACCGGTGACGGTGTGGAACCTCAGG
 T S G G T A A L G C L V K D Y F P E P V T V S W N S G
 1419 CGCCCTGACCAGCGCGTGCACACCTCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTACCG
 A L T S G V H T F P A V L Q S S G L Y S L S S V V T
 1499 TGCCCTCCAGCAGCTGGCACCCAGACCTACATCTGCAACGTGAACATCAGCAAGCCAGAACACCAAGGTGGACAGAAA
 V P S S S L G T Q T Y I C N V N H K P S N T K V D K K
 1579 GTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTGCTGGAAAGCCAGGCTCAGCGCTCTGCCTGGACGCATCCGGCT
 V
 1659 ATGCAGCCCCAGTCCAGGGCAGCAAGGCAGGCCAGGTCTGCCCTTCACCCGGAGGCCACTCATGCTC
 1739 AGGGAGAGGGTCTTCTGGCTTTTCCCAAGGCTCTGGCAGGCACAGGTAGGTACGTGCCCTAACCCAGGCCCTGCACACAA
 45 1819 AGGGCCAGGTGCTGGCTCAGACCTGCCAAGGCCATATCCGGAGGCCCTGACCTAACCCCACCCCAAGGC
 1899 CAAACTCTCCACTCCCTCAGCTGGACACCTCTCTCCAGATTCCAGTAACCTCCAACTTCTCTGCAGAGCCC
 E P
 1979 AAATCTTGTGACAAAACCTCACACATGCCAACCGTGCCAGGTAAAGCCAGGCCAGGCCCTGCCCTCCAGCTCAAGGCCAGG
 K S C D K T H T C P P C P
 50 2059 CAGGTGCCCTAGACTAGCCTGCATCCAGGGACAGGCCAGGCCCTGCCAGGTGCTGACACGTCCACCTCCATCTTCTCAGCA
 A
 2139 CCTGAACCTCTGGGGACCGTCAGTCTTCTCTCCCTCCAAAACCCAAAGGACACCCCTCATGATCTCCGGACCCCTGA
 P E L L G G P S V F L F P P K P K D T L M I S R T P E
 2219 GGTACATCGCTGGTGGACGTCAGGCCACGAAGACCCCTGAGGTCAAGTCAACTGGTACGTGGACGGCGTGGAGGTGC
 V T C V V V D V S H E D P E V K F N W Y V D G V E V
 2299 ATAATGCCAAGACAAGCCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCCTCACCGTCCCTGCACCAAG
 H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q
 2379 GACTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAA

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D W L N G K E Y K C R V S N K A L P A P I E K T I S K
2459 AGCCAAAGGTGGGACCGTGGGTGCGAGGCCACATGGACAGAGGCCGCTGGCCACCCCTCTGCCCTGAGAGTGACC
A K
2539 GCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCCACAGGTGTACACCTGCCCCCATCCGGATGAGCTGAC
5 G Q P R E P Q V Y T L P P S R D E L T
2619 CAAGAACCGGTCAAGCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATGCCGTGGAGTGGAGAGCAATGGC
K N Q V S L T C L V K G F Y P S D I A V E W E S N G
2699 AGCCGGAGAACAACTACAAGACCAACGCCCTCCCGTGCCTGGACTCCGACGGCTCCCTCTACAGCAAGCTCACCGT
Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V
10 2779 GACAAGAGCAGGTGGCAGCAGGGAAACGTCTCTCATGCTCGTGTATGCACTGAGGCTCTGCACAACCACTACACGAGAA
D K S R W Q G N V F S C S V M H E A L H N H Y T Q K
2859 GAGCCTCTCCCTGTCTCCGGTAATGAGTGCAGCGCCGGCAAGCCCCGCTCCCGGCTTCGGTCGACAGGA
S L S L S P G K
2939 TGCTTGACCGTACCCCTGTACATACTTCCCGGGCAGCATGGAAATAAA _GCACCCAGCGCTGCCCTGGGCCCTG
15 3019 CGAGACTGTGATGGTCTCTTCCAGGGCTAGGGCAGTCTGAGGCTGTGGCATGAGGGAGGGAGGCCAGGGGGTCCACT
3099 GTCCCCACACTGGCCAGGTGCTGAGGTGCTGGCTAGGGGGCTAGCCAGGGCTGCCCTGGCAGGG
3179 TGGGGGATTGCAAGCGTGGCCCTCCCTCCAGCAGCACGCTGGCTGGGCAACGGGAGGCCCTAGGAGCCCCGG
3259 GACAGACACACAGCCCTGCCTCTGTAGGAGACTGTCTGTGTGAGGCCCTGTCTCCGACCTCCATGCCCACTCG
3339 GGGGCATGCCTAGTCATGTGCGTAGGGACAGGCCCTCCCTCACCCATCACCCCCACGGCACTAACCCCTGGCTGCCCT
20 3419 GCCCAGCCTCGCACCGCATGGGACACAACCGACTCGGGGACATGCACTCTCGGGGCTGTGGAGGGACTGGTCAGA
3499 TGCCCCACACACACACTCAGGCCAGCCCTCAACAAACCCGACTGAGGTTGGGGCCACACGGGCCACACACAC
3579 ACGTGCAAGCCTCACACGGGACTCAGGGGGCAACTGACACGCCAGCACAGGAGCAAGGTCTCGCACAGTGA
3659 AACACTCTCGGACACAGGCCCCCACGAGGCCACCGCAGGCCACCTCAAGGCCACAGGCCCTCGGAGCTTCTCCACATGC
3739 TGACCTGCTCAGACAAACCCAGCCCTCTCACAGGGTGCCCCCTGCAGGCCACACACACAGGGGATCACACACC
25 3819 ACGTCACGTCCCTGGCCCTGGCCACTTCCCAGTGCCGCCCTCCCTGCAGGATCC (SEQ ID NO:16)

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Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably from immortalized B-cells. Suitable source cells for the polynucleotide sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources well-known in the art.

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). These polypeptide fragments may be produced by proteolytic cleavage of intact antibodies by methods well known in the art, or by inserting stop codons at the desired locations in vectors using site-

directed mutagenesis, such as after CH1 to produce Fab fragments or after the hinge region to produce F(ab')₂ fragments. Single chain antibodies may be produced by joining VL and VH with a DNA linker.

As stated previously, the polynucleotides will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences.

E. coli is a prokaryotic host useful particularly for cloning the polynucleotides of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a number of well-known promoters may be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. *Saccharomyces* is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention. Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, Syrian Hamster Ovary cell lines, HeLa cells, preferably myeloma cell lines, transformed B-cells, human embryonic kidney cell lines, or hybridomas.

Expression vectors for these cells can include expression control sequences, such as an

origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma

5 Virus, cytomegalovirus and the like.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic

10 cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Once expressed, the antibodies can be purified according to standard procedures, including ammonium sulfate precipitation, ion exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel electrophoresis, and the like.

15 Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or prophylactically, as directed herein.

The antibodies (including immunologically reactive fragments) are administered

20 to a subject at risk for or exhibiting A_{Beta}-related symptoms or pathology such as clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical amyloid angiopathy, using standard administration techniques, preferably peripherally (*i.e.* not by administration into the central nervous system) by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or

25 suppository administration. Although the antibodies may be administered directly into the ventricular system, spinal fluid, or brain parenchyma, and techniques for addressing these locations are well known in the art, it is not necessary to utilize these more difficult procedures. The antibodies of the invention are effective when administered by the more simple techniques that rely on the peripheral circulation system. The advantages of the

30 present invention include the ability of the antibody to exert its beneficial effects even though not provided directly to the central nervous system itself. Indeed, it has been

demonstrated that the amount of antibody that crosses the blood-brain barrier is $\leq 0.1\%$ of plasma levels.

The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners.

The concentration of the humanized antibody in formulations may range from as low as about 0.1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, and so forth, in accordance with the particular mode of administration selected. Thus, a pharmaceutical composition for injection could be made up to contain in 1 mL of phosphate buffered saline from 1 to 100 mg of the humanized antibody of the present invention. The formulation could be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250 mL of fluid, such as sterile Ringer's solution, and 1-100 mg per mL, or more in antibody concentration. Therapeutic agents of the invention can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 4 and 8 is tolerated.

Although the foregoing methods appear the most convenient and most appropriate for administration of proteins such as humanized antibodies, by suitable adaptation, other techniques for administration, such as transdermal administration and oral administration may be employed provided proper formulation is designed.

In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen.

In summary, formulations are available for administering the antibodies of the invention and are well-known in the art and may be chosen from a variety of options.

Typical dosage levels can be optimized using standard clinical techniques and will be dependent on the mode of administration and the condition of the patient.

5 The following examples are intended to illustrate but not to limit the invention.

The examples hereinbelow employ, among others, a murine monoclonal antibody designated "10D5" which was originally prepared by immunization with a peptide composed of residues 1-28 of human A β peptide. As the examples here describe experiments conducted in murine systems, the use of murine monoclonal antibodies is 10 satisfactory. However, in the treatment methods of the invention intended for human use, humanized forms of the antibodies with the immunospecificity corresponding to that of antibody 10D5 are preferred.

Example 1

Synthesis of Humanized Antibody 10D5

Cells and antibodies. Mouse myeloma cell line Sp2/0 was obtained from ATCC (Manassas, VA) and maintained in DME medium containing 10% FBS (Cat # SH30071.03, HyClone, Logan, UT) in a 37°C CO₂ incubator. Mouse 10D5 hybridoma cells were first grown in RPMI-1640 medium containing 10% FBS (HyClone), 10 mM 20 HEPES, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 25 μ g/ml gentamicin, and then expanded in serum-free media (Hybridoma SFM, Cat # 12045-076, Life Technologies, Rockville, MD) containing 2% low Ig FBS (Cat # 30151.03, HyClone) to a 1.5 liter volume in roller bottles. Mouse monoclonal antibody 10D5 (Mu10D5) was purified from the culture supernatant by affinity chromatography 25 using a protein-G Sepharose column. Biotinylated Mu10D5 was prepared using EZ-Link Sulfo-NHS-LC-LC-Biotin (Cat # 21338ZZ, Pierce, Rockford, IL).

Cloning of variable region cDNAs. Total RNA was extracted from approximately 10⁷ hybridoma cells using TRIzol reagent (Cat. # 15596-026, Life Technologies) and poly(A)⁺ RNA was isolated with the PolyATract mRNA Isolation System (Cat. # Z5310, 30 Promega, Madison, WI) according to the suppliers' protocols. Double-stranded cDNA was synthesized using the SMARTTMRACE cDNA Amplification Kit (Cat. # K1811-1, Clontech, Palo Alto, CA) following the supplier's protocol. The variable region cDNAs

for the light and heavy chains were amplified by polymerase chain reaction (PCR) using 3' primers that anneal respectively to the mouse kappa and gamma chain constant regions, and a 5' universal primer provided in the SMARTTMRACE cDNA Amplification Kit. For VL PCR, the 3' primer has the sequence:

5

5' -TATAGAGCTCAAGCTTGGATGGTGGGAAGATGGATAACAGTTGGTGC-3'

[SEQ ID NO:13]

with residues 17- 46 hybridizing to the mouse Ck region. For VH PCR, the 3' primers
10 have the degenerate sequences:

A G T
5' -TATAGAGCTCAAGCTCCAGTGGATAGACCGATGGGCTGTCGTTTGGC-3'
T

15 [SEQ ID NO:14]

with residues 17 - 50 hybridizing to mouse gamma chain CH1. The VL and VH cDNAs
were subcloned into pCR4Blunt-TOPO vector (Cat. # 45-0031, Invitrogen, Carlsbad, CA)
for sequence determination. DNA sequencing was carried out by PCR cycle sequencing
20 reactions with fluorescent dideoxy chain terminators (Applied Biosystems, Foster City,
CA) according to the manufacturer's instructions. The sequencing reactions were
analyzed on a Model 377 DNA Sequencer (Applied Biosystems).

Construction of humanized 10D5 (Hu10D5) variable regions. Humanization of the mouse antibody V regions was carried out as outlined by Queen et al., 1989, *op. Cit.* The human V region framework used as acceptor for Mu10D5 CDRs was chosen based on sequence homology. The computer programs ABMOD and ENCAD [Levitt, M., J. 5 Mol. Biol. 168:595-620 (1983)] were used to construct a molecular model of the variable regions. Amino acids in the humanized V regions that were predicted to have contact with CDRs were substituted with the corresponding residues of Mu10D5. This was done at residue 98 in the heavy chain and at residues 41 and 51 in the light chain. The amino acids in the humanized V region that were found to be rare in the same V-region subgroup 10 were changed to the consensus amino acids to eliminate potential immunogenicity. This was done at residues 42 and 44 in the light chain and at residue 24 in the heavy chain.

The light and heavy chain variable region genes were constructed and amplified using eight overlapping synthetic oligonucleotides ranging in length from approximately 65 to 80 bases [He, X. Y., et al., J. Immunol. 160: 1029-1035 (1998)]. The 15 oligonucleotides were annealed pairwise and extended with the Klenow fragment of DNA polymerase I, yielding four double-stranded fragments. The resulting fragments were denatured, annealed pairwise, and extended with Klenow, yielding two fragments. These fragments were denatured, annealed pairwise, and extended once again, yielding a full-length gene. The resulting product was amplified by PCR using the Expand High Fidelity 20 PCR System (Cat. # 1 732 650, Roche Molecular Biochemicals, Indianapolis, IN). The PCR-amplified fragments were gel-purified and cloned into pCR4Blunt-TOPO vector. After sequence confirmation, the VL and VH genes were digested with MluI and XbaI, gel-purified, and subcloned respectively into vectors for expression of light and heavy chains to make pV_k-Hu10D5 and pV_{g1}-Hu10D5 [Co, M. S., et al., J. Immunol. 25 148:1149-1154 (1992)]. The mature humanized 10D5 antibody expressed from these plasmids has the light chain of SEQ ID NO:11 and the heavy chain of SEQ ID NO:12.

Stable transfection. Stable transfection into mouse myeloma cell line Sp2/0 was accomplished by electroporation using a Gene Pulser apparatus (BioRad, Hercules, CA) at 360 V and 25 µF as described (Co, et al., 1992, *op. cit.*). Before transfection, pV_k-Hu10D5 and pV_{g1}-Hu10D5 plasmid DNAs were linearized using FspI. Approximately 30 10⁷ Sp2/0 cells were transfected with 20 µg of pV_k-Hu10D5 and 40 µg of pV_{g1}-Hu10D5. The transfected cells were suspended in DME medium containing 10% FBS and plated

into several 96-well plates. After 48 hr, selection media (DME medium containing 10% FBS, HT media supplement, 0.3 mg/ml xanthine and 1 µg/ml mycophenolic acid) was applied. Approximately 10 days after the initiation of the selection, culture supernatants were assayed for antibody production by ELISA as shown below. High yielding clones 5 were expanded in DME medium containing 10% FBS and further analyzed for antibody expression. Selected clones were then adapted to growth in Hybridoma SFM.

Measurement of antibody expression by ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc, Naperville, IL) were coated with 100 µl of 1 µg/ml goat anti-human IgG, Fc γ fragment specific, polyclonal antibodies (Cat. # 109-005-098, Jackson ImmunoResearch, West Grove, PA) in 0.2 M sodium carbonate-bicarbonate buffer (pH 9.4) overnight at 4°C. After washing with Washing Buffer (PBS containing 0.1% Tween 20), wells were blocked with 400 µl of Superblock Blocking Buffer (Cat # 37535, Pierce) for 30 min and then washed with Washing Buffer. Samples containing Hu10D5 were appropriately diluted in ELISA Buffer (PBS containing 1% 10 BSA and 0.1% Tween 20) and applied to ELISA plates (100 µl per well). As a standard, humanized anti-CD33 IgG1 monoclonal antibody HuM195 (Co, *et al.*, 1992, *op. cit.*) was used. The ELISA plate was incubated for 2 hr at room temperature and the wells were 15 washed with Washing Buffer. Then, 100 µl of 1/1,000-diluted HRP-conjugated goat anti-human kappa polyclonal antibodies (Cat # 1050-05, Southern Biotechnology, Birmingham, AL) in ELISA Buffer was applied to each well. After incubating for 1 hr at room temperature and washing with Washing Buffer, 100 µl of ABTS substrate (Cat #s 20 507602 and 506502, Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. Color development was stopped by adding 100 µl of 2% oxalic acid per well. Absorbance was read at 415 nm using an OPTImax microplate reader (Molecular 25 Devices, Menlo Park, CA).

Purification of Hu10D5. One of the high Hu10D5-expressing Sp2/0 stable transfectants (clone #1) was adapted to growth in Hybridoma SFM and expanded to 2 liters in roller bottles. Spent culture supernatant was harvested when cell viability reached 10% or below and loaded onto a protein-A Sepharose column. The column was 30 washed with PBS before the antibody was eluted with 0.1 M glycine-HCl (pH 2.8), 0.1 M NaCl. The eluted protein was dialyzed against 3 changes of 2 liters of PBS and filtered through a 0.2 µm filter prior to storage at 4°C. Antibody concentration was determined

by measuring absorbance at 280 nm ($1 \text{ mg/ml} = 1.4 A_{280}$). SDS-PAGE in Tris-glycine buffer was performed according to standard procedures on a 4-20% gradient gel (Cat # EC6025, Novex, San Diego, CA). Purified humanized 10D5 antibody is reduced and run on an SDS-PAGE gel. The whole antibody shows two bands of approximate molecular weights 25 kDa and 50 kDa. These results are consistent with the molecular weights of the light chain and heavy chain, or with the molecular weight of the chain(s) comprising a fragment, calculated from their amino acid compositions.

Example 2

10 *In vitro* binding properties of humanized 10D5 antibody

The binding efficacy of humanized 10D5 antibody, synthesized and purified as described above, was compared with the mouse 10D5 antibody using biotinylated mouse 10D5 antibody in a comparative ELISA. Wells of a 96-well ELISA plate (Nunc-Immuno plate, Cat # 439454, NalgeNunc) were coated with 100 μl of β -amyloid peptide (1-42) in 15 0.2 M sodium carbonate/bicarbonate buffer (pH 9.4) (1 $\mu\text{g/mL}$) overnight at 4°C.

After washing the wells with phosphate buffered saline (PBS) containing 0.1% Tween 20 (Washing Buffer) using an ELISA plate washer, the wells were blocked by adding 300 μl of SuperBlock reagent (Pierce) per well. After 30 minutes of blocking, the wells were washed with Washing Buffer and excess liquid was removed.

20 A mixture of biotinylated Mu10D5 (0.4 $\mu\text{g/ml}$ final concentration) and competitor antibody (Mu10D5 or Hu10D5; starting at 1000 $\mu\text{g/ml}$ final concentration and serial 3-fold dilutions) in ELISA Buffer were added in triplicate in a final volume of 100 μl per well. As a no-competitor control, 100 μl of 0.4 $\mu\text{g/ml}$ biotinylated Mu10D5 was added. As a background control, 100 μl of ELISA Buffer was added. The ELISA plate was 25 incubated at room temperature for 90 min. After washing the wells with Washing Buffer, 100 μl of 10 $\mu\text{g/ml}$ HRP-conjugated streptavidin (Cat # 21124, Pierce) was added to each well. The plate was incubated at room temperature for 30 min and washed with Washing Buffer. For color development, 100 $\mu\text{l}/\text{well}$ of ABTS Peroxidase Substrate (Kirkegaard & Perry Laboratories) was added. Color development was stopped by adding 100 $\mu\text{l}/\text{well}$ of 30 2% oxalic acid. Absorbance was read at 415 nm. The absorbances were plotted against the log of the competitor concentration, curves were fit to the data points (using Prism) and the IC₅₀ was determined for each antibody using methods well-known in the art.

The mean IC₅₀ for mouse 10D5 was 23.4 µg/mL (three separate experiments, standard deviation = 5.5 µg/mL) and for humanized 10D5 was 49.1 µg/mL (three separate experiments, standard deviation = 11.8 µg/mL). A second set of three experiments was carried out, essentially as described above, and the mean IC₅₀ for mouse 10D5 was 5 determined to be 20 µg/mL (SD = 1 µg/mL) and for humanized 10D5, the IC₅₀ was determined to be 16 µg/mL (SD = 0.6 µg/mL). On the basis of these results, we conclude that humanized 10D5 has binding properties that are very similar to those of the mouse antibody 10D5. Therefore, we expect that humanized 10D5 has very similar *in vitro* and *in vivo* activities compared with mouse 10D5 and will exhibit in humans the same effects 10 demonstrated with mouse 10D5 in mice.

Example 3

In vitro binding properties of mouse and humanized antibodies 10D5

Antibody affinity (KD = Kd / Ka) was determined using a BIACore biosensor 2000 15 and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-mouse Ig or anti-human Ig) was coupled via free amine groups to carboxyl groups on flow cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG was coupled to flow cell 1 as a background control. Monoclonal antibodies were captured to yield 300 resonance 20 units (RU). Amyloid-beta 1-40 or 1-42 (Biosource International, Inc.) was then flowed over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the chip, bound anti-Aβ antibody was eluted from the chip using a wash with glycine-HCl (pH 2). A control injection containing no amyloid-beta served as a control for baseline subtraction. Sensorgrams demonstrating association and dissociation phases were 25 analyzed to determine Kd and Ka. The affinity (KD) of mouse antibody 10D5 for Aβ 1-40 was determined to be 390 nM, and the affinity of humanized 10D5, prepared essentially as described in Example 1, was determined to be 209 nM. Affinity for Aβ 1-42 was biphasic for both mouse 10D5 and humanized 10D5. For mouse 10D5, the affinities for Aβ 1-42 were 0.57 nM and 4950 nM. Humanized 10D5 had affinities for 30 Aβ 1-42 of 0.19 nM and 1020 nM.

Example 4Epitope mapping of mouse and humanized 10D5

The BIACore is an automated biosensor system for measuring molecular interactions [Karlsson R., *et al. J. Immunol. Methods* 145:229-240 (1991)]. The advantage of the BIACore over other binding assays is that binding of the antigen can be measured without having to label or immobilize the antigen (i.e. the antigen maintains a more native conformation). The BIACore methodology was used to assess the binding of various amyloid-beta peptide fragments to either mouse 10D5 or humanized 10D5 (prepared substantially as described in Example 1). All dilutions were made with HEPES-buffered saline containing Tween 20. A single concentration of a variety of fragments of human A β or mouse A β 1-40 (BioSource International) was used. Human amyloid beta fragments 1-10 and 1-20 bound to mouse 10D5 and to humanized 10D5, while human A β fragments 10-20 and 16-25 did not bind to either antibody. Neither mouse 10D5 nor humanized 10D5 bound mouse A β 1-40. Using this methodology, the binding epitope for both mouse and humanized 10D5 appears to be between amino acids 1 and 10 of human A β .

Example 5In vivo experiments with 10D5

Unless otherwise stated, all studies used PDAPP mice, and all injections were intraperitoneal (i.p.) In general, a control group of mice received injections of saline. In some cases, another control group received injections of a non-specific mouse IgG preparation.

Six weeks of weekly injection of 360 μ g of 10D5 in old mice (24 month) raised soluble A β_{total} in hippocampus by 16% and A β 1-42 in hippocampus by 21%, while lowering hippocampal insoluble A β_{total} by 24% and A β 1-42 by 26% (no statistically significant difference; 9 animals per control group and 10 animals per antibody group). In the cortex, mean insoluble A β_{total} was lower by 27% and A β 1-42 by 29%, while mean insoluble A β 1-40 increased by 7% (no statistically significant differences).

In hemizygous, 4 month old mice, administration of 360 μ g 10D5 per animal: 1) raised average plasma A β 1-40 and A β 1-42 levels approximately 3-fold by 24 hours after administration; and 2) had no significant effect on soluble A β 1-40 in the cortex after 24

hours compared with saline control (no differences were statistically significant; 5 animals per group).

Administration of 360 µg of 10D5 per animal (5 animals per group, saline control): 1) raised average plasma A β 1-40 and A β 1-42 levels approximately 14-fold and 5 19-fold, respectively by 24 hours after administration; 2) had no consistent or significant effect on soluble or insoluble A β 1-40, A β 1-42, or A β _{total} in the cortex or hippocampus after 24 hours; 3) lowered soluble A β 1-40, A β 1-42, and A β _{total} in the cerebellum by 10 50% (p<0.05), 33%, and 13%, respectively; and 4) lowered insoluble A β 1-40, A β 1-42, and A β _{total} in the cerebellum by 53% (p<0.001), 46% (p<0.001), and 30% (p<0.01), respectively.

In young mice, administration of 360 µg of 10D5 per animal (5 per group): 1) raised average plasma A β 1-42 levels approximately 33% by 24 hours after administration; and 2) in the cortex, raised soluble A β 1-40 3.4-fold (p<0.001), lowered soluble A β 1-42 by 22% (p<0.05), lowered insoluble A β 1-40 about 10% and increased 15 insoluble A β 1-42 about 12%.

We claim:

1. Humanized 10D5 antibody.
2. A humanized antibody, or fragment thereof, comprising a humanized light chain comprising three light chain complementarity determining regions (CDRs) from the mouse monoclonal antibody 10D5 and a light chain variable region framework sequence from a human immunoglobulin light chain; and a humanized heavy chain comprising three heavy chain CDRs from the mouse monoclonal antibody 10D5 and a heavy chain variable region framework sequence from a human immunoglobulin heavy chain; wherein the light chain CDRs have the following amino acid sequences:

10 light chain CDR1:

1 5 10 15
Arg Ser Ser Gln Asn Ile Ile His Ser Asn Gly Asn Thr Tyr Leu Glu
(SEQ ID NO:1)

15 light chain CDR2:

1 5
Lys Val Ser Asn Arg Phe Ser (SEQ ID NO:2)

light chain CDR3:

20 1 5
Phe Gln Gly Ser His Val Pro Leu Thr (SEQ ID NO:3)

and the heavy chain CDRs have the following amino acid sequences:

heavy chain CDR1:

25 1 5
Thr Ser Gly Met Gly Val Ser (SEQ ID NO:4)

heavy chain CDR2:

30 1 5 10 15
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser
(SEQ ID NO:5)

and, heavy chain CDR3:

35 1 5 10
Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr (SEQ ID NO:6).

3. A humanized antibody or fragment thereof comprising a humanized light chain variable region having the sequence of SEQ ID NO:7 and a humanized heavy variable region having the sequence of SEQ ID NO:8.

4. The humanized antibody or fragment thereof of claim 3 having a light chain variable region of the sequence given by SEQ ID NO:9 and a heavy chain variable region given by SEQ ID NO:10.

5. The humanized antibody or fragment thereof of claim 3 having a light chain of the sequence given by SEQ ID NO:11 and a heavy chain of the sequence given by SEQ ID NO:12.

6. An antibody fragment obtainable by enzymatic cleavage of the humanized antibody of any one of claims 1 - 5.

7. An Fab or F(ab')₂ fragment of any one of the humanized antibodies of
10 claims 1 - 5.

8. The F(ab')₂ fragment of claim 7.

9. The Fab fragment of claim 7.

10. The humanized antibody or fragment of any one of claims 1 - 9, which is a single chain antibody.

15 11. The humanized antibody or fragment of any one of claims 1 - 10 that is an IgG₁ immunoglobulin isotype.

20 12. The humanized antibody or fragment of any one of claims 1 - 11, wherein the antibody or fragment thereof is produced in a host cell selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

13. A polynucleotide compound, comprising a sequence coding for the light chain or the heavy chain of the humanized antibody of any one of claims 1 - 12, or a fragment thereof.

14. A polynucleotide sequence, which when expressed in a suitable host cell, yields an antibody of any one of claims 1 – 12.

15. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 17, and a polynucleotide comprising a sequence that codes for the light chain variable region given by SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO: 11.

16. The polynucleotide of claim 13 or 14 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:18, and a polynucleotide comprising a sequence that codes for the heavy chain variable region given by SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:12.

17. An expression vector for expressing the antibody of any one of claims 1 - 12 comprising the polynucleotide sequence of any one of claims 13 - 16.

18. A cell transfected with the expression vector of claim 17.

19. A cell transfected with two expression vectors of claim 17, wherein a first vector comprises the polynucleotide sequence coding for the light chain and a second vector comprises the sequence coding for the heavy chain.

20. A cell that is capable of expressing the humanized antibody or fragment of any one of claims 1 – 12.

21. The cell of any one of claims 18 – 20, wherein the cell is selected from the group consisting of a myeloma cell, a chinese hamster ovary cell, a syrian hamster ovary cell, and a human embryonic kidney cell.

22. A pharmaceutical composition comprising the humanized antibody or fragment of any one of claims 1 – 12, and a pharmaceutically acceptable excipient.

23. A method of treating Down's syndrome, clinical or pre-clinical Alzheimer's disease, or clinical or pre-clinical cerebral amyloid angiopathy in a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

5 24. A method to inhibit the formation of A_β plaque in the brain of a human subject, comprising administering to the human subject an effective amount of the humanized antibody or fragment of any one of claims 1 – 12.

10 25. A method to reduce A_β plaque in the brain of a human subject, comprising administering to the human subject an effective amount of a humanized antibody or fragment of any one of claims 1 – 12.

26. The method of either of claims 24 – 25, wherein the subject is diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

15 27. The method of any one of claims 24 – 25, wherein the subject is not diagnosed with clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

20 28. Use of the humanized antibody or a fragment thereof according to any one of Claims 1 – 12 for the manufacture of a medicament, including prolonged expression of recombinant sequences of the antibody or antibody fragment in human tissues, for treating clinical or pre-clinical Alzheimer's disease, Down's syndrome, or clinical or pre-clinical cerebral amyloid angiopathy.

29. Use of the humanized antibody or fragment of any one of claims 1 – 12 for the manufacture of a medicament for treating Alzheimer's disease.

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10/19

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Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
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<210> 13

<211> 46

<212> DNA

<213> DNA primer

<400> 13
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46

<210> 14

<211> 50

<212> DNA

<213> DNA primer

<400> 14

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50

<210> 15

<211> 1953

<212> DNA

<213> humanized antibody

<400> 15

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120

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180

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240

accgattttc tggggtccca gacaggttca gtggcagtgg atcaggaca gatttcacac

300

tcaagatcg cagagtggag gctgaggatg tggagtttta ttactgcttt caaggttcac

360

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420

taatcttagaa attctaaact ctgaggggtt cgatgacgt ggccattctt tgcccaaagc

480

atttagtttta ctgcaaggc agaaaagcat gcaaaaggct cagaatggct gcaaaagagct

540

ccaaacaaaac aattttagaac tttattaagg aataggggga agcttaggaag aaactcaaaa

600

catcaagatt taaaataacgc ttcttggtct cttgtata attatctggg ataagcatgc

660

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720

gaactttgtt acttaaacac catccgtttt gttctttcc tcaggaactg tggctgcacc

780

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840

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900

cctccaatcg ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta

960

cagcctcagc agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc

1020

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1080

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1140

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<210> 16

<211> 3256

<212> DNA

<213> humanized antibody

<400> 16	
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cttccctgca ggatcc 3256

<210> 17

<211> 714

<212> DNA

<213> humanized antibody

<400> 17
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<210> 18

<211> 1416

<212> DNA

<213> humanized antibody

<400> 18
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<210> 19

<211> 238

<212> PRT

<213> humanized antibody

<400> 19

Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
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Ser Ser Ser Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
20 25 30

Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile
35 40 45

Ile His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Gly Thr Lys Val
115 120 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 20

<211> 472

<212> PRT

<213> humanized antibody

<400> 20

Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
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Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys
20 25 30

Pro Thr Glu Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
35 40 45

Ser Thr Ser Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
50 55 60

Ala Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
65 70 75 80

Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys
85 90 95

Ser Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala
100 105 110

Thr Tyr Tyr Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala
115 120 125

Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
130 135 140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145 150 155 160

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
165 170 175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
180 185 190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
195 200 205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
210 215 220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225 230 235 240

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
245 250 255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
260 265 270

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
275 280 285

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
290 295 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305 310 315 320

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
325 330 335

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
340 345 350

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
355 360 365

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
370 375 380

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
385 390 395 400

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
405 410 415

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
420 425 430

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
435 440 445

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
450 455 460

Ser Leu Ser Leu Ser Pro Gly Lys
465 470